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RICHARD ENGLISH VAUGHAN, 1884-1952

James G. Dickson

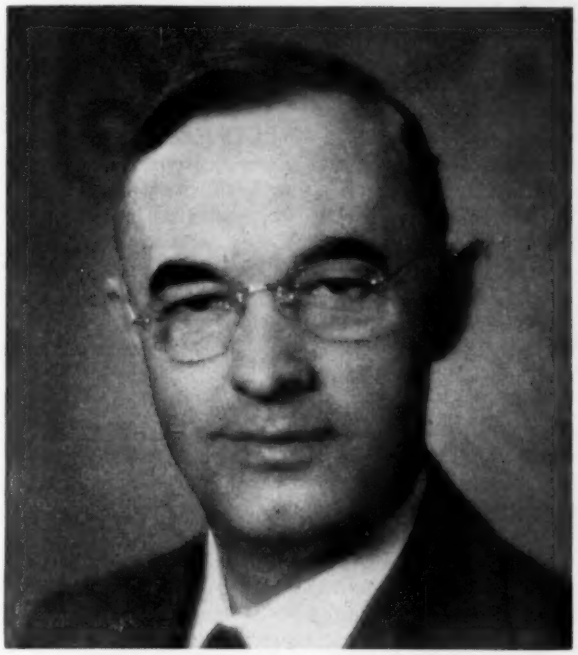
Richard English Vaughan, Emeritus Professor of Plant Pathology, died suddenly on October 6, 1952 in Madison.

Son of George Harlow and Frances Montague Vaughan, Richard was born at Woodstock, Vermont, March 27, 1884. He received his early education in Woodstock, where he graduated from High School in 1902. Undergraduate training at the University of Vermont under Dr. L. R. Jones and in close association with Professor William Stuart, culminated in 1907 with a B.S. degree *cum laude* in Agriculture, with a major in Agricultural Botany and Horticulture. He attended the summer school in agriculture at Cornell University in 1908. Majoring in Plant Pathology, he served as graduate assistant at the University of Wisconsin where he received the M.S. degree in 1912. He continued graduate study at Wisconsin, with a semester at Missouri Botanical Garden until 1914.

A long and successful career in agricultural adult education, soon to be known as agricultural extension, was started in Vermont in 1907. He was deputy inspector of feeds and fertilizers in the Vermont Department of Agriculture from 1904-1907. In April, 1906 he was assistant on the horticultural demonstration car of the first extension demonstration train run in the New England States. While teaching at the D. L. Moody's School for boys at Mt. Hermon, Massachusetts, from 1909 to 1910, he organized and assisted agricultural clubs in the area.

Professor Vaughan was one of the first extension plant pathologists in the United States. He and M. F. Barrus, Professor Emeritus, Cornell University, were the country's first extension pathologists under the Smith-Lever Act. In 1914 he was appointed Instructor in Plant Pathology at the University of Wisconsin on extension funds administered by the state. The following year he became Assistant Professor of Plant Pathology and in charge of Extension Work on Plant Disease Control. He was promoted to Associate Professor in 1919, to Professor in 1926. In 1931 he was made Extension Plant Pathologist, United States Department of Agriculture, and held the combined title—Professor of Plant Pathology and Extension Plant Pathologist—until his retirement in January 1950. He was a Collaborator in The Division of Mycology and Plant Disease Survey, United States Department of Agriculture, from 1914 to 1950.

His contributions in teaching and extension were numerous during his 38 years of continuous service to the state. He organized the first short course for canners, in 1923. He taught plant disease control to boys and girls in the Agricultural Short Course from 1918 until his retirement. His plant disease extension activities were coordinated closely with the programs in horticulture and agronomy. He cooperated harmoniously with the County Agricultural Agents and teachers in the agricultural high schools.



RICHARD ENGLISH VAUGHAN
1884–1952

He held membership in the American Phytopathological Society (life member), Botanical Society of America, Vermont and Wisconsin Horticultural Societies, Alpha Zeta, Phi Beta Kappa, Gamma Alpha, Sigma Xi, Epsilon Sigma Phi, and Acacia. He was active in church, fraternal, and civic affairs to the time of his death.

On September 15, 1914 he married Eloise Samson of Indianola, Iowa, a dynamic helper in the field of adult education. Besides his wife, he is survived by their 3 children, James Sampson Vaughan of Detroit, Michigan, Edwin Arthur Vaughan, of Hawthorne, New Jersey, and Marjorie Vaughan Ferris of Keokuk, Iowa, and 4 grandchildren.

Richard Vaughan was devoted to the dissemination of agricultural information to the farmers, helping them find an understanding of their plant disease problems and encouraging local adaptation of the best control methods. Pioneering in the new science of plant pathology, he was ever alert to take its findings to the farmers, working with and through the rapidly expanding extension personnel in the County Agent system, the agricultural teachers, and the leading farmers of the state. The University and the people of the state owe a debt of gratitude for the service he gave so freely.

His numerous publications were timely articles to growers, county agents, and others on plant disease control.

FOREST MILO BLODGETT, 1885-1951

M. F. Barrus, W. H. Burkholder and W. A. Rawlins

The sudden death of Forest Milo Blodgett on the morning of June 11, 1951 brought to an untimely close a long career of service to Cornell University and to agriculture. Surviving are his wife, Elsa James Blodgett, 3 children, and 5 grandchildren.

Professor Blodgett was born and reared on a farm near Brocton, New York. After a preparatory education at Stockton High School and Fredonia Normal, he attended Cornell where he graduated in 1910 with a degree of Bachelor of Science in Agriculture. As an undergraduate, he studied under the late Professor H. H. Whetzel who induced him to undertake graduate work in the field of plant pathology. He spent the next 4 years in graduate study as a Hermann Frasch research fellow and received his doctorate in 1914.

During the year 1914-1915, Professor Blodgett was Associate Botanist at the New York (Geneva) Agricultural Experiment Station, but returned to Cornell in 1915 to become an Assistant Professor in the Department of Plant Pathology. Subsequently, he was raised to the rank of Professor.

Professor Blodgett was a member of Sigma Xi, American Association for the Advancement of Science, American Phytopathological Society, and the Potato Association of America, serving the latter association as vice-president in 1940 and president in 1941.

In the early years of his professional life, Professor Blodgett devoted his time to research on the control of hop mildew and apple diseases. He is better known, however, for his extensive work and writings concerning virus and fungus diseases of the potato; on the control of scab by disinfectants, soil amendments, and agronomic practices; on the use of sprays and dusts for potato pest control; and on the development of potato varieties resistant to scab. He originated the tuber-index method of testing tubers for the presence of virus diseases, and improved the method for estimating losses due to missing hills or to virus infection. Blodgett was a student and ardent advocate of modern biometrics as a means of increasing the worth of field and laboratory experimentation. His strict obedience to biometrical practice, always a pattern for his research, earned for him a reputation for sound conservative judgment.

Quiet and unassuming, Professor Blodgett had no propensity for classroom or extension teaching. The influence of Professor Blodgett can in the final analysis be estimated only in the large number of graduate students who majored under his guidance and by the many students, as well as colleagues, who constantly sought his advice on a host of varied subject matter. He leaves behind a rich heritage in his many students well trained under his guidance in the field of plant pathology.

The titles of some of Professor Blodgett's publications are as follows:

1911. Studies on the fungicidal value of lime-sulphur preparations. New York (Cornell) Agr. Exp. Sta. Bul. 290. (With E. Wallace and L. R. Walker).

1913. Hop mildew. New York (Cornell) Agr. Exp. Sta. Bul. 328.

1914. Experiments in the dusting and spraying of apples. New York (Cornell) Agr. Exp. Sta. Bul. 340.

1917. Dusting as a substitute for spraying. Proc. New York State Fruit Grower's Assoc. **16:** 61-75.

1919. Further developments in the work on mosaic and leaf-roll of potatoes. Proc. New York State Pot. Assoc. **6:** 9-11.

1921. Testing seed potatoes for mosaic and leaf-roll. Phytopathology **11:** 58-59. (With K. Fernon).

1923. Time temperature curves for killing potato tubers by heat treatments. Phytopathology **13:** 465-475.

1927. Tobacco mosaic on potatoes. Phytopathology **17:** 727-734.

1928. A preliminary experiment using calomel as a dip treatment of seed potatoes. Amer. Pot. Jour. **5:** 6-12.

1934. Factors influencing the occurrence of potato scab in New York State. Cornell Agr. Exp. Sta. Bul. 581. (With F. B. Howe).

1934. A method of recording the distribution of copper dusts or sprays on leaves. Phytopathology **24:** 418-422. (With E. O. Mader).

1938. The spread of apple mosaic. Phytopathology **28:** 937-938.

1940. A second report on the effect of agronomic practises on the incidence of Rhizoctonia and scab of potatoes. Amer. Pot. Jour. **17:** 290-295.

1941. A method for the determination of losses due to disease or missing plants. Amer. Pot. Jour. **18:** 132-135. (With P. Decker and C. S. Turhill).

1946. The new scab-resistant potatoes, Ontario, Seneca and Cayuga. Amer. Pot. Jour. **23:** 315-329. (With F. J. Stevenson).

DEFENSE REACTIONS OF PLANTS TO THE PRESENCE OF TOXINS [1]

Robert Bloch

The title suggested for this paper seems to rest on 2 assumptions: first, that toxins occur in plants; second, that there are defense reactions to the presence of such toxins. The first of these assumptions may stand or fall, depending on how we interpret the meaning of the word toxin. The justification to speak of defense reactions will to some extent depend on our willingness to include forms of antipathogenic behavior in plants which might not exactly exhibit that kind of specificity which serves as a criterion for the antitoxic reactions in the animal and human body.

The problem itself may be approached in various ways. The questions as to how toxins may be formed by pathogens and how their presence within the host tissue may be proven beyond doubt are under examination at the present time by workers qualified in these fields. A knowledge of wound reactions and specific pathologic-anatomical and physiological changes is also important; some of these are discussed here. Significant results may be forthcoming soon which should be of value both to the practical plant pathologist to whom plant disease constitutes a challenge and to the student of atypical growth and morphogenesis who is concerned with problems of the relationship between normal and atypical growth processes in the plant body.

In plant pathology the toxin theory of disease has gained great momentum only recently. Many disease symptoms as well as histological defense reactions in plants are attributed to some sort of interference of pathogens with the typical metabolism and growth of the host. It has been customary to speak in such event of toxic action or of toxic effects. Such usage is noncommittal as regards the mechanism since there may be involved toxins produced by the pathogen, toxic substances released by necrobiotic or necrotic cells of the host, and non-specific physiological changes. In some cases pathogenic toxins have been extracted or their presence demonstrated *in vitro* and identical effects produced by injection into a normal host (6, 10, 14, 20). It is difficult, however, to distinguish *within* the host tissue pathogen-produced toxins from host-reaction products. In general the toxic action is simply inferred because we associate it with the syndrome of certain systemic diseases, such as vascular wilt, or with morphological and chemical changes in the host cells. These include necrotic degeneration, secondary processes of auto-intoxication by substances produced by the host cells themselves, in and near the infected area, and cell division and growth (2, 3, 8, 9). It will be important in the future to determine exactly the nature and sequence of these events. The presence of pathogenic toxins in the infected cells, the actual manner of their production, their spread, the method by which the destruction of host cells is accomplished, and the true nature of the so-called decomposition products in those cells which are in a state of necrosis and necrobiosis should be investigated. We especially want to know about the relationships between toxic cellular reaction products and wound or growth hormones and about their effect on either the pathogen or on healthy cells. Defense reactions are an expression of the coordinated, regulative activity of the plant. This is complex in itself, and perhaps more so in specific host-parasite relationships of great physiological subtlety. Our imagination may easily become focused on one or another of the more readily discernible aspects of factorial interplay, and the experimental analysis becomes difficult when such a multiplicity of causal schemes is involved.

In a comparison of defense reactions in plants with those in animals, it appears that plants possess a high general resistance to pathogens, but that the body of humans and animals is organized in a superior way for dealing with individual instances of infection. Plant pathology, physiology, and morphogenesis share their own problems in coming to grips with biochemical specificities. The bodies of most animals are endowed with an efficient circulatory system, with definite kinds of hormonal control, specific types of irreversible cellular determination, and, in case of infection, with characteristic immunity reactions. In plants the activity of the hormones, auxin, and indole acetic acid is rather non-specific, while more specific hormones are known to us only by circumstantial evidence. Plants lack the mobile defense cells of the animal; nothing is known quite as general and typical as the systemic antigen-antibody reaction, not to speak at all of that expression of high organization in the animal body which manifests itself in the capability of responding to different kinds of antigens in just the proper and competent manner.

Nature of defense reactions.—Generally genetic and environmental factors as well as mechanical-anatomical, chemical, and physiological properties may not only determine resistance to infection, but also be instrumental in defense. When a defense barrier is formed, either to the spread of the pathogen or its toxic effects, we can make a list of gross anatomical and chemical changes in the host cells, and sometimes we can demonstrate *in vitro* (11, 12) the actual antibiotic properties of compounds suspected of being deleterious to the pathogen. There are familiar kinds of defense reactions which may be described with some assurance; they are associated with various local lesions, necrosis, cellular hypertrophy, hyperplasia and gall formation. The difficulties are much greater, however, when we have to explain reaction differences in

[1] An invitational paper presented at the Symposium on Physiology of Parasitism at the 44th Annual Meeting of The American Phytopathological Society in Ithaca, N. Y., September 9, 1952.

the host toward different biotypes of the same pathogen (5, 8). In such subtle differences of host-parasite relationship and physiological specialization we may assume that there are yet undefined kinds of defense reactions, not visible to the eye and not so readily detected microchemically.

Even visible symptoms of defense are not too well understood. We distinguish the shock-like necrotic reactions of the hypersensitive type of host cell from reactions that proceed more slowly and involve a greater number of cells (8, 9). In the former case an invading parasite of the obligate, biotrophic kind may be weakened and eliminated speedily, and the necrotic area may remain quite limited. In the latter case there may be much necrobiosis; gummy decomposition products of host cells may infiltrate intercellular spaces, ducts, and lifeless skeleton cell material; there is usually meristematic activity around such areas. In both categories it will be necessary to determine whether, besides the breakdown products of the host cells, there may be present toxic emanations of the pathogen itself. It will be interesting to examine at what stage such toxins enter the picture. Only then will we be able to know whether the changes in the host cells are direct reactions to the action of the pathogen or its toxins, and will it be possible to establish a meaningful terminology. According to Gäumann (8, 9), anti-infectional reactions are related to necrotic changes at the focus of infection, anti-toxic reactions to necrotic changes at a distance. But pathogen-produced toxins could well evoke anti-toxic reactions in both the pathogen-containing cells and in cells further distant.

In the more extensively studied pathological wilts, we deal with a very complex situation. A parasite, such as *Fusarium*, produces certain toxins in the roots and lower portion of the plant. In the region of infection the effect will be a local tissue necrosis. But toxins also enter the vascular system and are transported to more distant parts of the plant where they produce the wilting symptoms. Gäumann and his collaborators have analyzed the situation for *Fusarium* wilt of tomato (10). They have suggested that the pathogens causing infectious wilt diseases produce their effects by means of 2 chemically distinct kinds of toxins: peptides and quinones. *Fusarium lycopersicum*, for example, produces a polypeptide wilting toxin, lycomarasmin. This toxin has a coagulating effect on the protoplasm of the host cells by which plasmatically bound water is released, leading to an excessive rate of transpiration; it also affects the semipermeability of the plasma membrane, resulting in turgor change. Other kinds of toxins are glucosans which mechanically block the intermicellular spaces of the cell walls and the ends of the vascular bundles.

DEFENSE REACTIONS AND WOUND REACTION.—We know from experiments which include a great variety of treatments, such as simple wounding, mechanical pressure, application of injurious inorganic and organic chemical substances that plants respond to a great many nonspecific kinds of irritation with the same or very similar degenerative cellular changes (2, 3). These somehow induce in adjacent resting cells a highly activated state which results in increased metabolic activity, in dedifferentiation and growth responses. This has the practical result that the necrotic area becomes cut off from the rest of the tissue by a meristem of the nature of periderm. If the initiating agent of these growth processes had been a pathogen, this barrier, together with the necrotic zone proper, frequently constitutes an efficient histogenetic defense. Sometimes meristematic activity does not occur, namely in those cases when the reactivity of the tissue cells is low, and the barrier will be formed from cells only which have undergone some secondary change due to the transport from more distant regions of locally formed decomposition products. If such infiltration occurs only in cells dead even before the infection occurred, calling such a reaction a defense reaction becomes indeed only a matter of convenience.

Considering, however, the typical course of events, the crucial question would appear to lie in the following fact. The toxic action of the pathogen and the general wound reaction which has the character of defense may be actually 2 separate processes which overlap, and thus we can not be certain at the present that the 2 stand in a specific relationship to each other. What is this wound reaction? Until about a decade ago it has been fairly intensely studied so that some, though by no means sufficient, information is available concerning the physiology and chemistry of wound healing (2, 3). We know, for example, about changes in permeability, respiratory activity, microchemical and metabolic changes, and other factors. Research in this field has slackened, presumably because the isolation from wounded cells of what were believed to be specific compounds acting as cell division or wound hormones convinced many that at least the problem of wound healing had been brought to a satisfactory solution. Such an assumption would constitute, however, a gross oversimplification. The wound hormones are thought to be oxidation or "decomposition" products of substances normally present in uninjured cells. Traumatic acid (a dicarboxylic acid) appears to be one of them, and possibly also indole acetic acid. The wound reaction is indeed of a complex nature: after the physiological balance of the living cells has been upset sufficiently by injurious, though non-specific, agents of various kinds, one can observe a combination of morbid processes and meristematic activity. In other words, there are substances formed which have been called intracellular toxins, necrohormones, etc.; these appear, on the one hand, to promote the death of cells, on the other hand, to be instrumental in the division of other healthy cells. It often looks, judging from distance effects, as though the type of response, (cell death or cell division) which these products evoke, depended simply on their concentration, but there is no good quantitative evidence. In the formation of the cork meristem or periderm in aging plant parts we actually see the 2 proc-

esses in some way interlinked, without artificial, external injury of the cells involved. The possibility should not be excluded that more exact information concerning the mechanism of the wound reaction and the role of the wound toxins may throw light on the manner and place of action of pathogen-produced toxins as well.

CHEMICAL ASPECTS OF DEFENSE.—The protective action provided by the necro- and histogenic demarcation zone is probably complex. We know nothing of what the wound toxins may do to the toxins of the pathogen. The parasite itself, unless it is of the type that is able to use dead cell material, may be mechanically impeded, or it may perish on account of substances toxic to it. In such case of resistance, the presence of the germicidal substances may often be demonstrated in the noninfected plant. However, in infected plants the necrotic, gummy material may vary to some extent with the type of pathogen present, especially after a stage of disease is reached (19). Especially alkaloids and phenols have been shown to exert germicidal effects. A well-known example is that studied by Walker (25). Certain brown-skinned onion varieties are resistant to smudge (*Colletotrichum circinans*), owing to the presence in the tissue of protocatechuic acid and catechol. Another example is the characteristic resistance series of *Phymatotrichum omnivorum* to different phenolic compounds and alkaloids (11, 12). According to Johnson and Schaal (15), resistance to common scab (*Streptomyces scabies*) of potato varieties is closely related to the presence in the superficial layer, especially in and near the lenticels, of chlorogenic acid, a phenolic compound, and possibly also of tyrosinase. Chlorogenic acid or its quinone also accumulate in the periderm and around injuries whether mechanical or parasitic in origin.

There is fairly good evidence that resistance to leaf rust in durum and einkorn wheats and relatively high susceptibility in common wheats are correlated with the presence or absence of phenolic compounds (16, 17) and wound gum (22, 24).

It would be entirely feasible to design experiments in which by means of nonspecific wounding and other injuries the tissue cells would be made to change chemically in such a way that they are rendered resistant to attack by certain pathogens and thus to the spread of their toxins. Wounding may also have typically systemic effects as in wilt diseases. Keyworth and Dimond (18) injured tomato roots, before inoculating them with *Fusarium*. Three methods were used: dipping into hot water, periodical pruning, or applying sub-lethal doses of root-damaging compounds. These various treatments had in common the nonspecific type of root injury, which resulted in reduced disease severity. The injured plants had a higher soluble carbohydrate and lower protein, calcium and phosphorus content.

DEFENSE REACTION AND PATHOGEN.—Whether the necrotic wound recation is advantageous to the plant depends, of course, on the type of pathogen causing the reaction. To facultative or necrotrophic invaders the disintegrating host cells furnish the substrate upon which they strive. On the other hand, the reactions assume defensive character in relation to parasites of the biotrophic or obligate type which do not utilize dead substance.

In this way certain varieties of clover are resistant to mildew since their epidermal cells become necrotic on contact with the hyphae of the fungus *Erysiphe* (23). Another biotrophic pathogen is *Synchytrium endobioticum*. Those potato varieties are resistant whose cells react hypersensitively and develop necrosis and gummy degeneration whereby this parasite is killed. In certain varieties the necrosis spreads around the infected cells and the whole area becomes delimited by a meristem. *Botrytis cinerea* induces the same kind of necrosis, but, being a facultative parasite, is not itself affected.

The resistance to biotrophic rusts of varieties of cereals that develop necrogenous defense reactions is well-known. Certain wheats are resistant to species of *Puccinia* (1), similarly hypersensitive Victoria oats to *Puccinia coronata avenae*. But these oats are, as shown by Meehan and Murphy (21), highly susceptible to the toxins of the facultative parasite *Helminthosporium victoriae*. Both pathogens produce, according to Litzenberger (20), toxins which result in comparable hypersensitive reactions. The growth of *Puccinia coronata*, an obligate parasite, is checked by the ensuing necrosis, while the growth of the facultative parasite *Helminthosporium victoriae* is facilitated by it.

The list may be closed with 3 characteristic instances of histogenic and gummous defense reactions. The first, a typical histogenic reaction, is the shot hole disease of stone fruit trees which is caused by the conidia of *Clasterosporium carpophilum* (*Coryneum beijerinckii*). Infection, for example in *Prunus amygdalus* Stok., is followed by cell necrosis, inducing adjacent cells to divide and to form a typical demarcation tissue. Similar demarcation reactions occur in other leaf spot diseases, for example, in leaves of *Citrus limonia* attacked by *Sphaceloma fawcettii*, and in ivy attacked by *Phyllosticta hasicola*.

Peridermatous demarcation tissues are found also in foot and root rots of tobacco caused by *Thielavia basicola*. They also occur in the roots of flax varieties that are resistant to the wilt caused by *Fusarium lini*, and in the young roots of trees attacked by *Armillaria mellea*.

In general, however, reactions in trees are of the gummous variety as exemplified by the areas in pathological heart wood which contain gum and tannin. *Stereum purpureum* is a common parasite whose toxins are carried to the upper parts of deciduous trees with the sap, causing silver leaf. Those varieties of plum (4), especially the Pershore variety, which set up a barrier to the passage of the toxic products of substances containing gum and tannin are resistant.

There must be other defense reactions besides those

mentioned in this survey. Gross chemical changes do not explain the phenomena of physiological specialization, for instance, in wheat leaf rust. Gassner and Hassebrauk (7) have suggested that in susceptible forms species-specific, serologically distinct proteins may act as antitoxins to the toxins produced by the rust fungus. The cells would thus stay alive and the development of the pathogen be facilitated. Chester (5), on the other hand, expressed the view that such proteins may play the role of nutrients, the absence of which in resistant plants would starve the fungus which consequently would perish and kill the host cell.

Yet different forms of toxin-related behavior, such as the phenomena of premunity and of induced tolerance in virus-infected plants, should be mentioned. Likewise the important complex of research which has to do with the artificial antidoting of pathogenic toxins in virus, bacterial, and fungus diseases and in vascular wilts (13). The mechanism by which the various chemotherapeutic agents produce their effects is quite varied, but its discussion is outside the scope of this paper.

A problem somewhat related to those discussed here is that of gall formation. Why, we may wonder, does one sort of parasite induce in a given plant the formation of simple demarcation tissues, another one bring forth structures of highly organized and differentiated character and of determinate growth, yet another type, such as *Agrobacterium tumefaciens*, a tumor-like, amorphous, proliferating mass of tissue? In certain cases the last-named organism even induces a physiological, pseudo-cancerous change which is expressed morphologically in unrestricted tissue growth. In this last instance, too, we find the familiar sequence of pathological and morphogenetic phenomena, namely microbial toxic action, resulting in the death and necrosis of cells and concomitant meristem formation, but also a peculiar effect which amounts to interference with the mechanism controlling the growth-differentiation balance of the highly activated and dividing host cells. There is a good chance that the study of the physiology of parasitism may yet lead us to the doorstep where the mysteries of both normal and atypical development meet.

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AN APPROACH TO THE PHYSIOLOGY OF TOXIN FORMATION IN MICROORGANISMS [1]

John P. Hollis [2]

Many attempts have been made to advance knowledge of plant disease etiology from a microbial to a chemical level. General microbiological progress in recent years bears mute testimony to the faith of pioneer workers in the field of toxin[3] investigations. Developments in antibiotics research, particularly, have made it abundantly clear that the problem of etiology must be reworked completely in certain selected cases if we are to gain insight into fundamental disease processes.

To assess the etiological significance of a chemical substance it must be isolated from cultural filtrates or diseased tissues, purified and analyzed chemically. We must be able to detect and coordinate its presence in the plant in one form or another with the production of relevant symptoms in both the presence and the absence of the pathogen. Since a single plant pathogen may produce several toxic substances and since disease causation may involve phenomena not attributable to toxins, even in nonobligate organisms, the task is one of considerable magnitude. Further progress in such a field will be dependent upon the resolution of technical difficulties and the development of new approaches, and can be achieved only by coordinated attacks on all its phases.

Two approaches are essential to a sound toxin theory although they may not necessarily contribute proof of the etiological significance of a toxic substance. One of these involves the mechanistic aspects of toxin formation, the other, modes of action of toxins. Their recognition underscores some of the most fundamental questions in microbiology: how do pathogens differ from nonpathogens? How is it that antibiotics produced by microorganisms in artificial culture media do not appear to be involved generally in competitive processes among microorganisms in soil? Such questions emphasized recently by Gottlieb and his associates (10) illustrate the well-known but often neglected fact that biological interactions are dynamic phenomena. The biochemical methods leading to proof of chemical etiology provide a static picture of disease processes and must be supplemented by studies on mechanisms of formation and action of toxins.

A direct approach to the problem of the mechanism of toxin formation may be made by searching for precursor-product relations in cultural filtrates and diseased plant tissues but can be profitably pursued in specific instances only after considerable knowledge of chemical etiology has accumulated. For example, the use of this method has achieved notable success in the discovery that phenylacetic acid is a precursor of penicillin (17) but its value as a general tool in plant pathology is subject to certain limitations. Attempts to alter the output by a microorganism of an unknown toxic substance introduce elements of uncertainty. Serious complications are engendered by adding the host plant for bioassay purposes to a reaction complex which already includes the pathogen, toxins and modifying chemicals. Yet, the host must be present at one time or another because one can never be sure that the toxins produced in its absence are involved in pathogenesis, even though their chemistry be known. Alternatively, it might be supposed that correspondence of symptoms produced by the pathogen and its separate toxic filtrates can provide a way out of this dilemma. Unfortunately such is not the case because symptoms at best are generalized expressions of specific activity.

How then can a general approach be made to the problem of toxin formation in microorganisms which will avoid these methodological pitfalls? The answer is supplied by the brilliant work of Avery and his colleagues reviewed recently by Dubos (6). These workers discovered certain correlations between structure and pathogenicity in the pneumococci and other bacteria. Correlation analysis provides a tool for seeking relations between pathogenicity and other manifestations of a microorganism. Its strength lies in the fact that manifestations which are perfectly correlated with basic pathogenic capabilities are true and relevant differences amenable to further analysis.

In the present work the possibility of correlations between certain manifestations of a well known metabolic system and pathogenicity of a selected organism has been explored in terms of a toxin theory of disease. *Streptomyces scabies* (Thaxt.) Waks. and Henr. was selected as a test organism because of its wide host range (15), and particularly because of its melanin-indicator system (13). The production by this organism of local necrotic lesions on plants fostered the hope that complex systemic reactions such as occur in the fusarioses could be avoided. Its wide host range and ubiquity in soil gave promise that any toxins produced would possess correspondingly broad spectra of biological activity.

A simple mechanical model (Fig. 1A, B) is used to

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[3] While the historical distinctions which confer different meanings to the terms "toxin" and "antibiotic" have been adhered to, particularly in reference to specific literature, it should be recognized that there exist no fundamental chemical or biological criteria for such distinctions.

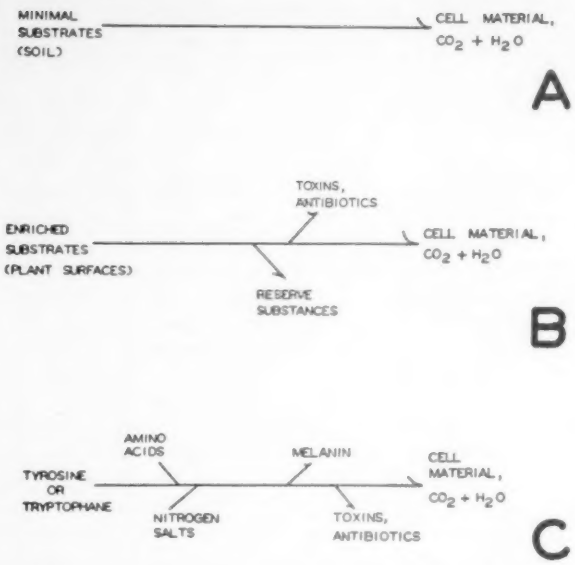

FIG. 1. Diagrams illustrating a shunt hypothesis of toxin formation. A) Efficient utilization of substrate; B) Inefficient utilization of substrate with formation of certain intermediate compounds; C) Production of melanin by *S. scabies* and a possible indirect relation between the melanin shunt and shunts of toxic substances in terms of stimulatory amino acids.

illustrate an idea of the formation of toxins in *S. scabies* by a metabolic shunt from the normal synthetic pathways. In the situation depicted by Fig. 1A all substances metabolized are utilized efficiently, producing maximal energy by complete oxidation to CO_2 and H_2O, whereas in Fig. 1B certain links in the synthetic pathways become overloaded due to substrate concentration or enzyme deletion caused by a gene block and intermediate compounds accumulate as illustrated by toxins, antibiotics and reserve substances.

Considering the chemical nature of some microbial toxins and antibiotics it is probable that the metabolism of the amino acids may be linked in one way or another with the formation of these compounds. In terms of carbon or nitrogen economy of stimulatory compounds there may be an indirect relation of shunts as illustrated in Fig. 1C. An example of shunt or overflow metabolism of an amino acid is furnished by the conversion of tyrosine to melanin by *S. scabies* under substrate conditions of tyrosine in supersaturation (0.1 per cent concentration) an inorganic nitrogen source such as KNO_3, favorable pH (neutral or alkaline) and the presence of stimulatory amino acids. The organism under these conditions converts more than 10 per cent of the tyrosine to melanin during a 4 day period in shake culture (14). Nitrogen content of this melanin averages about 10 per cent on the third and fourth days, a value corresponding closely to the percentage of nitrogen (9.79) in indole-5, 6-quinone, a melanic substance postulated by Mason and Wright (16) to be formed from dihydroxyphenylalanine, a derivative of tyrosine. Tryptophane is included in the diagram because it was found to stimulate pigment production by *S. scabies* in a medium similar to that described above (1).

Theoretically the production of toxins and antibiotics may be examples of shunt metabolism when the organism in question is brought into contact with unusual substrates such as plant surfaces or enriched culture media (7). Such responses to unusual substrates need not be regarded as "pathological" in nature. Indeed, present viewpoints in biochemistry emphasize the complexity and compensatory powers of the cell with literally hundreds of intermediate compounds known to be formed in "normal" metabolism. If any of these be released by the cell they might be regarded as shunted compounds provided they possess the potential advantage of falling partly into an alternative pathway. The shunt idea is not essential to the present approach but the mechanical models illustrated in Fig. 1 enable one to visualize a mechanism of increase in toxin production similar to that derived by Foster (7) to explain "acquired characters" such as the enhanced activity of mutants for production of antibiotics.

A possible interrelation in terms of stimulatory amino acids between the melanin shunt[4] and hypothetical toxigenic systems was sought. Glutamic acid, which stimulates melanin production, may be used to illustrate this idea. If this substance were to mediate in toxin production, its effect in stimulating the tyrosine-melanin shunt system might be impaired. Such impairment should result in alteration of the melanin-stimulation pattern of a particular isolate (Fig. 2) and would then constitute presumptive evidence of some role for this acid in toxin production.

EXPERIMENTAL METHODS.—One of the first steps was identification of the stimulatory substances. It was reported previously (11) that melanin production in *S. scabies* is stimulated by additions to a minimal synthetic medium of yeast extract, peptone, tryptone and acid and enzymatic casein hydrolysates and that the active principle is dialyzable from yeast extract (13). Acid casein hydrolysate was chosen for further study because of its relative chemical simplicity. Initial addition experiments with single amino acids, (L and DL isomers) indicated the effect could be achieved in different degrees by several individual acids of either type. In view of the fact that peptides in acid casein hydrolysate might possess activity also it was decided to separate this substance into 4 fractions: basic, acidic, aromatic, and neutral nonaromatic acids and peptides and to compare these fractions with appropriate mixtures of amino acids stimulating the fractions. Separation was achieved by 2 different chromatographic procedures (9, 23). In 1 series the basic, acidic and aromatic substances, respectively, were removed with silica gel, alumina, and charcoal, leaving the neutral nonaromatic substances. In the

[4] The melanin shunt system has been characterized (14).

other series, these same substances were removed with ion exchange resins, amberlites IRC-50, IR-4B and charcoal. Results of testing fractions from the 2 series were in fair agreement and the magnitude of stimulation brought about by each fraction was approximately equal to that actuated by the respective simulated fraction composed of a mixture of amino acids. It seemed reasonable to assume that any stimulatory effect of peptidic substances in acid casein hydrolysate was too small to be considered further at this time.

Single addition experiments with amino acids (L-isomers) were conducted in order to elucidate melanin-stimulation patterns of 2 isolates of *S. scabies*: a pathogenic isolate, #25, (Hooker's #5)[5], and a non-pathogenic isolate, #26, previously pathogenic, (Hooker's #9). Also tested was a saprophytic species of *Streptomyces* from a scab lesion surface.

Acids of low solubility were added to the medium as it was prepared. In most instances 1 per cent solutions were adjusted to pH 7.0 and molar equivalents equal to glycine in 0.1 per cent final concentration were added to Petri plates containing a synthetic medium which included 0.1 per cent tyrosine (13). Inoculation with the appropriate isolate was effected by spreading 0.05 ml. of a filtered spore suspension over the agar surface with a glass rod.

EXPERIMENTAL RESULTS.—The composite results of several experiments with isolate 25 are shown in Fig. 2A (14). The appearance of melanin in the plates in number of hours prior to its appearance in unsupplemented control plates was taken as the index of melanin-stimulation. Of the amino acids known to occur in acid casein hydrolysate, (8), serine and hydroxyglutamic acid were not tested. Arginine, lysine, histidine, aspartic and glutamic acids are markedly stimulatory. Phenylalanine, alanine, and proline are stimulatory to a minor degree while cystine and glycine prove to be inhibitory. The same acids are major stimulants for isolate 26, (Fig. 2B). Alanine, proline, valine, and leucine are of minor significance. The pattern for isolate 63, (Fig. 2C) shows the basic and acidic acids again are markedly stimulatory, with the exception of histidine, but including alanine. Histidine, phenylalanine, valine, and cystine exhibit minor effects. In summary, there exist differences in degree with the principal stimulatory acids and differences in kind among the minor acids but it is notable that the patterns are generally equivalent with regard to arginine, lysine, histidine, aspartic acid, glutamic acid, and 2 of less importance—phenylalanine and alanine.

[5] S. scabies #5 and #9 are isolates obtained from Dr. W. J. Hocker, Iowa State College. The author expresses his appreciation to Dr. Hooker for testing their pathogenicity.

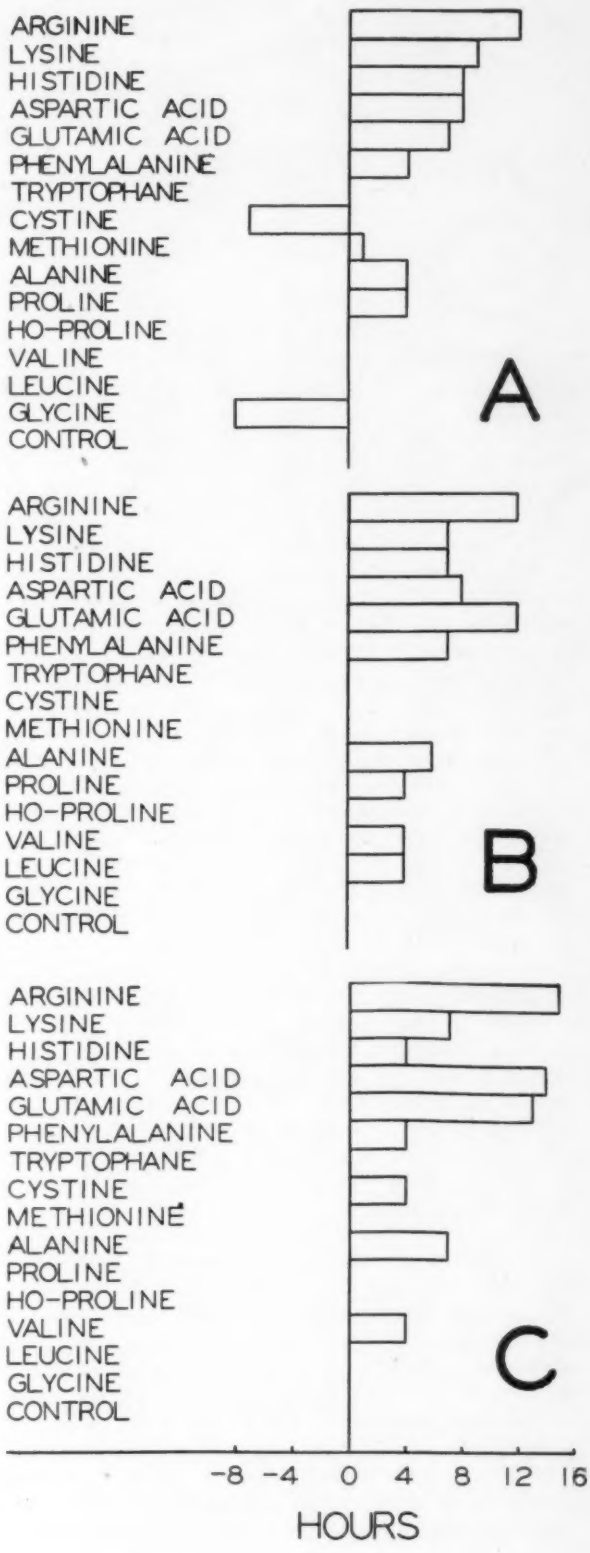

FIG. 2. Melanin-stimulation patterns of species of *Streptomyces*, with supplements of individual amino acids (L-isomers), showing the appearance of melanin in number of hours (+) before and (−) after its appearance in control plates. A) *S. scabies* #25; B) *S. scabies* #26; C) *S. sp.* #63. Figure A is reproduced (14).

TABLE 1.—*Comparisons between melanin-stimulation and growth-acceleration properties of amino acids (L-isomers) in S scabies (2 isolates) and a saprophytic species of Streptomyces. Data for isolate 25 are reproduced (14)*

Amino Acid	Melanin (hours) Isolate			Generation Time (minutes) Isolate		
	25	26	63	25	26	63
arginine	22	22	15	57	55	30
lysine	25	27	23	73	110	49
histidine	26	27	26	56	52	40
aspartic acid	26	26	16	53	68	38
glutamic acid	27	22	17	55	81	38
tyrosine	—	—	—	340	—	—
phenylalanine	30	27	26	142	166	75
tryptophane	34	34	30	150	162	82
cystine	41	34	26	145	165	112
methionine	33	34	30	140	180	72
alanine	30	28	23	160	140	55
proline	30	30	30	100	116	55
HO-proline	34	34	30	154	130	100
valine	34	30	26	210	170	95
leucine	34	30	30	124	190	95
glycine	42	34	30	172	170	80
control	34	34	30	147	170	110

Melanin production has been correlated with growth (13, 14). A detailed comparison of these variables was made with the 3 isolates, (Table 1). The data show the effects of the different acids in hours required for initial appearance of melanin compared with the generation time of growth in minutes on the same medium free of tyrosine (12). Growth and melanin-stimulating properties of the basic and acidic acids are correlated. The range of variation in generation times for the nonstimulatory acids as compared with the control for any 1 isolate is large and probably is not statistically significant. Of special interest is the marked inhibitory effect of tyrosine on growth of isolate 25 and the correspondence of this fact with the tyrosine-melanin shunt. In the case of isolate 63 there is a lack of correlation between the melanin and growth-stimulating properties of histidine. This acid is relatively weak as a stimulator of melanin production but ranks with the dicarboxylic acids in acceleration of growth. A few other examples of this lack of correlation may be seen but they are not as pronounced as the histidine effect.

DISCUSSION.—The recognized importance of amino acids in the phenomenon of parasitism has been highlighted in recent papers by Van Lanen, Riker and Baldwin (20, 21) and De Vay, Rowell and Stakman (5). The former authors showed that a number of monoamino monocarboxylic acids in addition to diglycine and lysine inhibited growth and attenuated virulence of crown gall bacteria. Their results stand at the forefront of our knowledge of amino acids and parasitism and implicate the present work by virtue of the inhibitory effects of glycine and cystine on isolate 25.

Cochrane and Peck (4) showed the respiratory pattern in *S. scabies* to be consistent with the operation of a tricarboxylic acid cycle. The known mediation of aspartic acid, glutamic acid, and alanine in this cycle through the formation of corresponding keto acids furnishes a tentative explanation for their stimulatory effects upon growth and melanin production (2). Speculation on the mechanism of stimulation by arginine, lysine, and histidine must await further work.

Application of melanin-stimulation patterns to the study of parasitism in *S. scabies* is dependent upon activation of the indicator system during the period of pathogenesis and upon its sensitivity to stimulatory compounds. Both melanin and toxin production must be correlated with growth and both must be activated within similar or overlapping ranges of pH in parasitized tissues.

The amino acid content of potato tissues and the increase in scab lesion pH 7.2 (19) should favor the production of melanin. A recent review by Brian (3), of studies on antibiotics production by fungi, indicates, in the majority of known cases, that production of these substances is correlated with growth. Furthermore, it was revealed that many antibiotics are produced in quantities ranging from 100–500 mg./l. of culture media. Additional tests of the melanin-producing ability of arginine with *S. scabies* showed that less than 1 part in 10,000 is required in the culture medium for measurable stimulation over the control. Presumably, toxic substances of small molecular weight would have to be produced in concentrations approximating the above figure in order to effect a measurable diversion of any one of the principal stimulatory acids from its role in melanin production to toxin formation.

The similarity in melanin-stimulation patterns among the 3 isolates can be interpreted in several ways: a) the amino acids tested do not mediate in parasitic activity, b) the sample of 3 isolates is inadequate to confirm small differences of degree or kind which may be of significance, or c) the differences may be revealed only by adaptive responses to certain unknown substrate constituents, such as those in soil extracts which stimulate parasitic activity of *S. scabies* (15).

The melanin-stimulation patterns presented above are built up from tests of individual amino acids, each exerting independent effects and such patterns taken as a whole are of static significance only. The rudimentary techniques used in this work are inadequate to the resolution of a dynamic pattern of amino acid activity. The relations which are being sought may be revealed only by simultaneous determinations of amino acid levels in the medium and in the cells as some of them mediate in melanin production and others play a role in toxin formation.

Further research with *S. scabies* should be directed at attempts to alter both melanin-stimulation patterns and pathogenicity by mutagenic agents on the one hand and by additions of evocative substances on the other. Tests of additional isolates and amino acids are indicated.

The artificial development of indicator systems useful in the study of toxin formation in other micro-

organisms is a distinct possibility and may be accomplished by 2 methods either singly or in combination. These are: a) overloading the synthetic metabolic pathways by increasing the substrate concentrations of relevant substances, b) alteration of metabolic pathways by the use of mutagenic agents; each result in the production of a metabolic shunt. An example of the first method is furnished by the present work and of the second by the results of Mitchell and Houlahan (18). These authors obtained a mutant of *Neurospora crassa* unable to synthesize adenine from adenine precursor. Blocked in normal outlet, the precursor polymerizes to form a purple pigment.

Results of this study point up the necessity of finding relevant functional correlations between pigment-producing and toxigenic systems before predictions can be made concerning the utility of this method as a general approach to the mechanistic aspects of parasitism.

It now appears that the possibility of such correlations can be most critically tested in certain chromogenic species of *Streptomyces* which produce well-known antibiotics. *S. lavendulae* (Waks. and Curt.) Waks. and Henr. (22) should be an excellent choice in this respect since its antibiotics contain nitrogen and are produced during growth and chromogenesis. While the application of such methods to this organism would probably produce information on the physiology of toxin formation that could be obtained more simply by precursor-product studies, it must be emphasized that in diseases the difficulty of toxin bioassay imposes an effective barrier to this approach. It is for this reason only that an indirect attack on the problem of functional correlations is justified.

For approximately 75 years students of parasitism have been attempting to differentiate between pathogenic and nonpathogenic microorganisms. As a result primarily of the efforts of Avery and his colleagues (6) correlations between pathogenicity and structure have been established in certain bacteria. The discovery, in generality, of functional correlations between pathogenicity and other physiological manifestations in microorganisms is yet to be achieved and remains a challenge to the present generation of workers.

SUMMARY

The possibility of an indirect relation, in terms of stimulatory compounds, between a tyrosine-melanin shunt and metabolic toxigenic systems was explored in *Streptomyces*.

Melanin production and growth in *Streptomyces* were stimulated by additions to a minimal tyrosine medium of the L-isomers of arginine, lysine, histidine, phenylalanine, alanine, aspartic, and glutamic acids. Both similarities and differences were found in the melanin-stimulation patterns of a pathogenic isolate of *S. scabies*, an isolate of *S. scabies* which had lost pathogenicity and a saprophytic species of *Streptomyces* from a scab lesion surface.

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THE GROWTH OF RUST IN TISSUE CULTURE[1]

Hugh Howison Hotson

Studies on physiological processes in the rust fungi are complicated because these microorganisms are obligate parasites. Thus the host tissue is involved in almost any investigation, but this host tissue has been either the entire plant with its complicated and diverse physiology, or fragments of the host tissue in the process of physiological degradation.

In an effort to study the physiology of the rust using host tissue which is actively metabolising, the tissue culture technique was the natural approach. By growing both infected and noninfected tissues of the host plant, under controlled conditions, the metabolism of the rust might be studied.

MATERIALS AND METHODS.—For the following study several types of rust infection were selected: A) a relatively localized infection, represented by *Melampsora lini* (Pers) Lév. race 210 on Bison flax; B) a systemic infection, represented by *Gymnoconia peckiana* (Howe) Trotter on *Rubus hispidus* L; C) a type causing a malformation of the host, represented by *Calyptospora columnaris* Kuhn on *Vaccinium* sp., and D) a type causing galls on the host, represented by *Gymnosporangium juniperi-virginianae* Schw. (cedar-apple rust) on *Juniperus virginiana L.*

The techniques used to induce callus formation in the selected hosts were those of Gautheret (2); the reader is referred thereto for a complete discussion.

The standard medium used for the initial isolation of tissue cultures was a modification of Gautheret's Medium IV:

Knop's solution composed of:

$Ca(NO_3)_2 \cdot 4H_2O$	0.5 gm./l.
KNO_3	0.125 gm./l.
$MgSO_4 \cdot 7H_2O$	0.125 gm./l.
KH_2PO_4	0.125 gm./l.

Dextrose CP 30 gm./l

Agar—Nobels or strip agar washed 24 hr. in tap water 10 gm./l.

Bertholot's solution for trace elements 0.5 ml./l

Cystiene 10 mg./l.

Yeast extract 0.5 gm./l.

Ascorbic acid 300 mg./l.

Naphthalene acetic acid at rates desired 10^{-6}, 5×10^{-7}, 10^{-7}, 10^{-8} grams/liter are usually tried initially

Water (double distilled in glass) to make 1 liter pH 5.1–5.2

The formula for Bertholot's solution is given on page 52 of Gautheret's book on tissue culture. In these experiments titanium chloride was substituted for titanium sulfate and cobalt nitrate for cobalt chloride on a mole for mole basis of Ti and Co.

The ferric chloride was omitted from Knop's solution when Berthelot's solution was added because of the high ferric sulfate content of the latter.

This medium was tubed in Pyrex tubes 2.5 cm. × 15.5 cm., in aliquots of 15–20 ml. per tube. The agar was allowed to harden with tubes in a vertical position.

The callus tissues were grown at 70°C. under constant white fluorescent illumination of 600 f.c.

RESULTS.—Callus formed on the healthy stem of Bison flax when naphthalene acetic acid (NAA) in concentration of 10^{-6}, 5×10^{-7}, 10^{-7}, 10^{-8} gm./l. was added to Gautheret's medium IV. In figure 1A and 1B, callus development after 6 weeks' growth on 10^{-7} and 5×10^{-7} gm./l. NAA is illustrated. As these cultures matured, a great many adventitious roots were formed just beneath the callus. These roots were most numerous on a medium with 5×10^{-7} gm./l. NAA, and when subcultured on liquid medium with 5×10^{-7} gm./l. NAA, they grew so rapidly as to fill the base of a 125-ml. Erlenmyer flask in 30 days. They were subcultured through 4 transfers and then discarded.

The calluses which developed on the medium with 10^{-7} gm./l. NAA were transferred after 60 days to a medium with 10^{-8} gm./l. NAA. They lost viability rapidly and all were dead within 150 days from the beginning of the experiment.

Concurrently calluses were induced on flax which was infected with *Melompsora lini* race 210. The surface for regeneration of callus tissue was selected by cutting through the uredium on the stem. Callus on this surface was induced in a medium with NAA at 10^{-7} gm./l. Twenty-five tubes were prepared on September 12, 1949, and allowed to develop until November 12, 1949, when they were transferred to media containing different quantities of NAA. Five calluses were transferred and supplied with NAA 10^{-8} gm./l., 5 with 5×10^{-8} gm./ l., and 5 with 10^{-7} gm./l.; 5 others were examined for development of rust into the callus, and 5 were transferred to fresh media without being removed from the stem piece. The transferred callus tissues lost viability rapidly and all were dead within 45 days. Those not removed from the stem continued to grow and produced a few roots, and teliospores were formed in the uredium. In no case, however, was there evidence of rust in the newly formed callus tissue.

On the same medium (NAA 10^{-7} gm./l.) regeneration of tissue was induced on the cut surface of twigs of *Vaccinium* sp. infected with *Calyptospora columnaris* (Figure 1C). Roots with very little callus tissue

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formed on the cut surface of the upper twig portion. The roots were very striking in that the central portion of the root was bright red while the outer layers of the root were hyaline. Callus tissue that formed from the stem tissue adjacent to the agar survived only 1 transfer. There was no histological evidence that the rust grew into this callus tissue. Lack of material prevented further experimentation.

Two species of *Rubus* have been previously cultured by Morel (4), and his technique was applied to the present work with *Rubus hispidus*. All but 1 of the 150 initial cultures with a systemic infection of *Gymnoconia peckiana*, died before the calluses could be transferred. This one (Fig. 1D) grew rapidly but had no evidence of the rust infection after a year and a half.

The gall of the cedar-apple rust (*Gymnosporangium juniperi-virginianae*) was the only tissue from which both infected and noninfected tissues were successfully grown. Noninfected tissue was obtained by cambium cultures from noninfected Juniper on medium with 5×10^{-7} gm./l. of NAA. Calluses were transferred, after initial formation, to medium with 10^{-7} gm./l. of NAA, and they continued to grow for a year and a half. The first efforts to grow the infected galls failed completely because dark melanin-like products were produced when the galls were exposed to the air. The addition of 150 mg. ascorbic acid to each liter of the medium remedied this, and the galls were able to grow.

To test the nature of this melanin-like formation, the galls were heated in water for 30 minutes at 85°C. and upon exposure to air, the melanin-like products were not formed, indicating that the system probably was enzymatic in nature. Those enzyme systems which use molecular oxygen as the electron acceptor are called oxidases. Burris and Little (1) limit the term oxidases to those enzymes which are metallo-protein enzymes reducing atmospheric oxygen to water and not forming hydrogen peroxide as an intermediate. They discuss the terminology and mode of action in their review of the subject. Nelson and Dawson (5) preferred the term tyrosinase; Burris and Little (1) also adopted this nomenclature. Robinson and Nelson (7) have shown that as long as reduced ascorbic acid is present in a potato or mushroom tyrosinase preparation, the solution remains colorless. This is also true of the galls of *Gymnosporangium*. When first cut and aseptically placed in a sterile solution of 1 per cent ascorbic acid, they remain orange and no melanin-like products are formed. They then can be placed on the medium containing ascorbic acid in tubes and grow. Apparently the exposure to molecular O_2 disrupts the reducing system in the cells.

The infected tissue soon outgrows its need for ascorbic acid or calluses over sufficiently to prevent the effect of O_2 because the tyrosine and dopa are not oxidized to melanin later on during growth.

One noticeable characteristic of these galls in the presence of beta and gama carotene as shown by

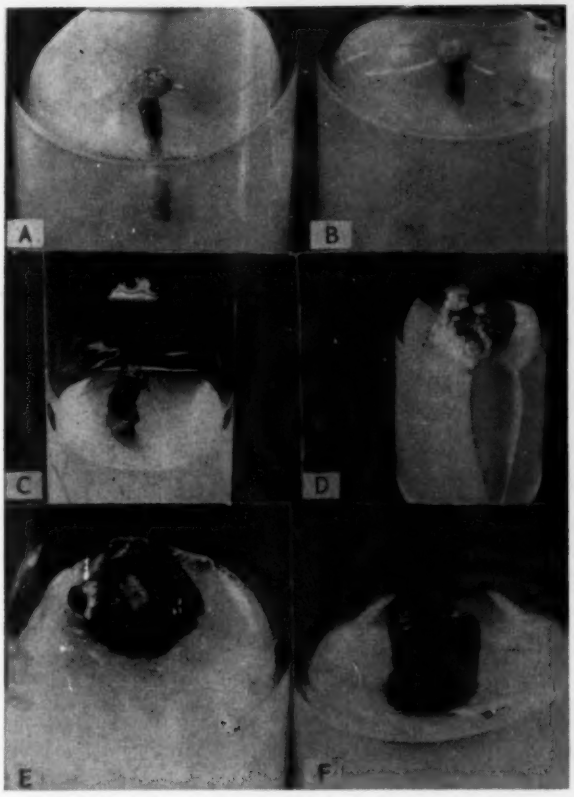

FIG. 1, A and B) Callus formation in Bisox Flax as affected by 2 concentrations of naphthalene acetic acid (A-NAA 10^{-7}; B-NAA 5×10^{-7}); C) Callus formation in *Vaccinium* sp. infected with *Calyptospora columnaris*, NAA 10^{-7}; D) Callus formation in *Rubus hispidus* infected with *Gymnoconia peckiana*, NAA 10^{-7}; E and F) Sporulation of *Gymnosporangium juniperi-virginianae* in tissue culture of *Juniperus virginianum*.

Smits and Peterson (8, 9). The telial horns produced on germination were particularly rich in carotene, but it also could be found in the developing gall itself.

Within 2 weeks after being placed on the media and grown under constant illumination at 600 f.c. at 70°F., the interior of the galls changed from orange to brilliant green.

The gall tissue grew very slowly. In an effort to increase the growth rate, the N:P:S rates were varied according to the nutrient triangle method described by Voth (10) and used by Riker and associates (3, 6). The standard triangular concentrations of N:P:S were as follows: (8:1:1), (6:1:3), (4:3:3), (3:1:6) and (3:6:1), in 4 replications each. All but 2 calluses grew very poorly and became yellow; 4 died. One callus only (4:3:3) remained green and one on (8:1:1) remained green. None grew so well as the controls on Gautheret's medium in which the N:P:S ratio is approximately 50:7:15. Therefore Gautheret's medium used as a standard basic medium was supplemented by 1) aqueous extracts of the gall tissue (15 per cent), 2) coconut milk (15 per cent), 3) yeast extract (.4 gm./l), 4) carrot juice (15 per cent), 5) tomato juice

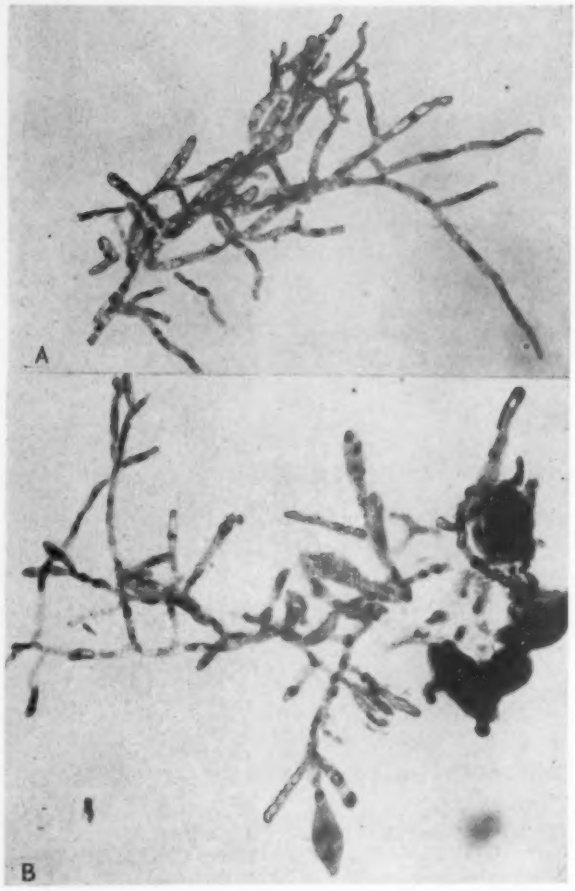

FIG. 2, A and B. Aerial mycelium of *Gymnosporangium juniperi-virginianae* stained by the Giemsa Technique (× 440).

(15 per cent), 6) orange juice (15 per cent), and 7) lemon juice (15 per cent). Growth was stimulated most by 1) yeast extract, 2) carrot juice, and 3) gall extract. None of the other ingredients were stimulatory. Tomato juice, orange juice, and lemon juice had definite toxic effects although titrated to pH 6 with 0.1 NaOH before autoclaving. This toxicity might have been due to salt concentration caused by titration, but it was not investigated further.

Thus the modification of Gautheret's basic medium IV was the best medium for the Juniper galls.

The gall extract was also tested for vitamin B_{12} activity against *Lactobacillus leishmannii*, but results were negative.

After the galls had been in culture approximately 4 months, rust activity was evidenced by active growth in the regions of the telia primordia, which appear as small discs on the surface of the gall. After 6 months, typical "horns" of the cedar-apple rust (Fig. 1E and 1F) appeared in the area of the primordial discs, as occurs in nature. At the base of the telial horns was a collar of aerial vegetative rust mycelium (Fig. 1E). On the mycelium adjacent to the collar, the teliospores were borne terminally on lateral branches, but the mycelium in the outer area was purely vegetative. The distinct binucleate condition of the mycelium is illustrated in Figure 2A and 2B. No clamp connections were found in this species.

When the telial horns were aseptically removed from the callus, teliospore production did not cease but the orientation into a columnar structure disappeared and the localization of teliospore production also was disarranged. The teliospores were no longer produced only in the vicinity of the primordial discs but appeared in short stalks over the entire surface of the callus on no organized pattern whatsoever.

As new callus grew from the galls, it appeared at first that the rust failed to grow into this newly formed tissue. Later several sub-culturable calluses apparently infected with rust were obtained. The fungus mycelium could be found in this new callus tissue by microscopic examination. This infected tissue as well as the healthy tissue has been continually sub-cultured since March, 1949. Since the rust has not sporulated in the sub-cultured calluses the evidence of its presence rests purely on the presence of fungus mycelium in the callus.

SUMMARY

Healthy callus tissue of *Juniperus virginana* has been grown in culture since March 1949.

Gall tissue from Juniper infected by *Gymnosporangium juniperi-virginianae* was grown in tissue culture. The rust produced typical telial horns, and a fungus mycelium survived in sub-cultures of the gall callus in serial transfers since March 1949.

Sub-cultures of the galls, free from rust, were grown from serial transfers since March 1949.

A "polyphenol" oxidase system probably functions in the galls of *Gymnosporangium juniperi-virginianae*. In the presence of ascorbic acid no melanin-like products were formed.

The aqueous extract of the *Gymnosporangium* galls does not contain vitamin B_{12} as it does not promote the growth of *Lactobacillus leischmannii* when used as bioassay material.

The vegetative mycelium of *Gymnosporangium juniperi-virginianae* growing aerially from the cultured host callus is binucleate, dichotomously branched, and without clamp connections.

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SOME FACTORS IN THE CONTROL OF THE SOUTHERN BLIGHT ORGANISM, SCLEROTIUM ROLFSII [1]

S. P. Johnson

Sclerotium rolfsii Sacc. incites the disease commonly known as southern blight, causing varying degrees of damage to a wide variety of crops. It infests the more acid, light sandy soils of the warmer temperate and tropical areas of the world.

Many have studied the organism and the disease it incites but few have discussed or recommended control methods. Higgins (5) reported that liming sweet potato slip-beds in Georgia gave good control. Leach and Davey (8, 9) in California recommended heavy applications of nitrogenous fertilizers as a method of reducing the sclerotial population in beet fields but stated that liming (9) had little, if any, effect. Langley (7) found applications of nitrogenous fertilizers to be of little benefit in reducing disease incidence in peanuts at Stephenville, Texas.

The present paper is concerned with results of an exploratory investigation of certain nutritional and physiological factors which may affect the control of *S. rolfsii*.

NUTRITIONAL STUDY.—Three common nitrogen fertilizer salts—$(NH4)_2SO_4$, $NaNO_2$, and NH_4NO_3; 1 calcium salt, $CaCl_2$; and nitrite as sodium nitrite were used in this study. The effect of each was determined by growing the organism on varied concentrations of the salt in liquid medium.

The basic solution used was that recommended by Joham (6), consisting of 0.0015 M. $MgSO_4$ or 0.0015 M. $MgCl_2$, 0.004 M. K_2HPO_4, 0.002 M. KCL, 0.1 p.p.m. thiamine chloride, and 4 or 8 per cent glucose. Micronutrients were not added directly as it was found that reagent grade salts and glucose furnished amounts as contaminents sufficient for good growth of the organism. The solutions were all adjusted to pH 6.5 prior to inoculation.

The isolate of *S. rolfsii* used was obtained from an infected watermelon fruit in 1951. Pure cultures of this isolate grown on watersoaked, autoclaved oats in 250-ml. Erlenmeyer flasks were used as inoculum.

For studies of the organism on liquid media 50-ml. portions of the nutrient solution were placed in 250-ml. Erlenmeyer flasks. The flasks were sterilized in steam in 121° C. for 20 minutes and inoculated with 1 infected oat grain per flask. Either 4 or 5 cultures were used for every combination of experimental conditions. The cultures were incubated at 30° C. until most of the sclerotia were mature, i.e. turned a dark brown. The fungal mats were then removed, washed free of surplus nutrient solution, and dried at 70° C. to constant weight.

S. rolfsii exhibited little inhibition of growth in the presence of extremely high levels of the nitrogen salts tested, with the exception of $NaNO_2$, (Fig. 1). The amounts employed were much higher than is normally present in soils under high levels of nitrogenous fertilization. Nitrite was found to be toxic at 20 p.p.m. with complete suppression of growth occurring at 400 p.p.m.

Restriction in growth of *S. rolfsii* cultured at various levels of ammonium nitrate and sodium nitrate did not become apparent at less than 20,000 p.p.m. concentration. Ammonium sulfate toxicity was observed at 40,000 p.p.m. concentration, or twice that of ammonium or sodium nitrate.

The inhibition of growth of *S. rolfsii* by calcium followed approximately the same pattern as that of the nitrogen fertilizer salts. Growth was maintained until the 4000 p.p.m. level was reached.

The nature of the suppression of growth is not known. The organism was able to maintain growth as the concentration of the salts increased until the critical level was reached, after which the mat weight fell off rapidly. In general, maturity dates increased from 15 days at the lowest levels (all salts) to 60 days at the highest (ammonium sulfate).

An examination of the sclerotia at all salt levels revealed no reduction in viability. However, it was noted that as the concentration of the salt increased, the number of sclerotia produced on the mycelial mat at maturity decreased. This reduction was very marked in the case of calcium chloride. Steinberg (10) has pointed out the essentiality of micronutrients for the growth and sporulation of fungi; hence it is probable that high concentrations of calcium interfered with

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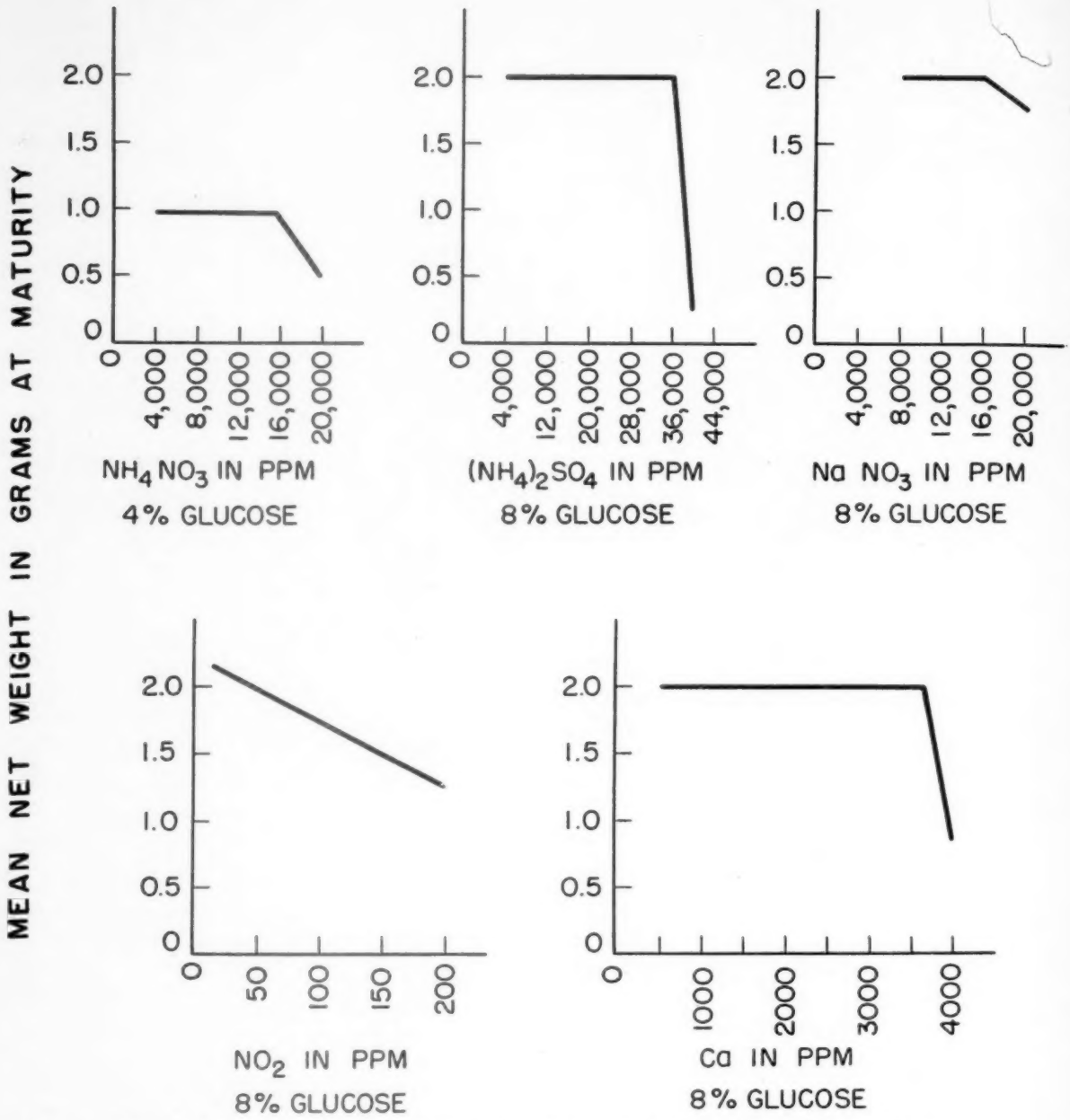

FIG. 1. Effect of different concentrations of inorganic salts on growth of *S. rolfsii* in Joham's solution.

micronutrient absorption and limited both growth and sclerotia formation.

SOIL MICROFLORA STUDY.—The work of Leach and Davey (8) showed that both ammonium and nitrate inorganic salts were capable of lowering the germination percentage of the sclerotia. Upon repeating this work, it was found that the results could be duplicated with single salts but not with a complete nutrient solution; i.e., one containing nitrogen, potassium, phosphorus, glucose, and thiamine chloride. It then became evident that the reduction of sclerotia in beet fields, as reported by Leach and Davy, might be due to some other factor, such as an increase in microfloral antagonism toward *S. rolfsii*. Accordingly, an experiment designed to study the effect of the soil microflora on the sclerotial state of *S. rolfsii* was conducted.

The soil selected, Lufkin fine sandy loam with a pH of 5.6, is low in organic matter and nitrogen. Fraps and Fudge (3) reported that a typical sample of the soil contained 0.065 per cent N, 0.033 per cent P, and 0.78 per cent K. Although they did not determine organic matter content, an estimated value of 1.39 per cent may be obtained by multiplying nitrogen by 20.

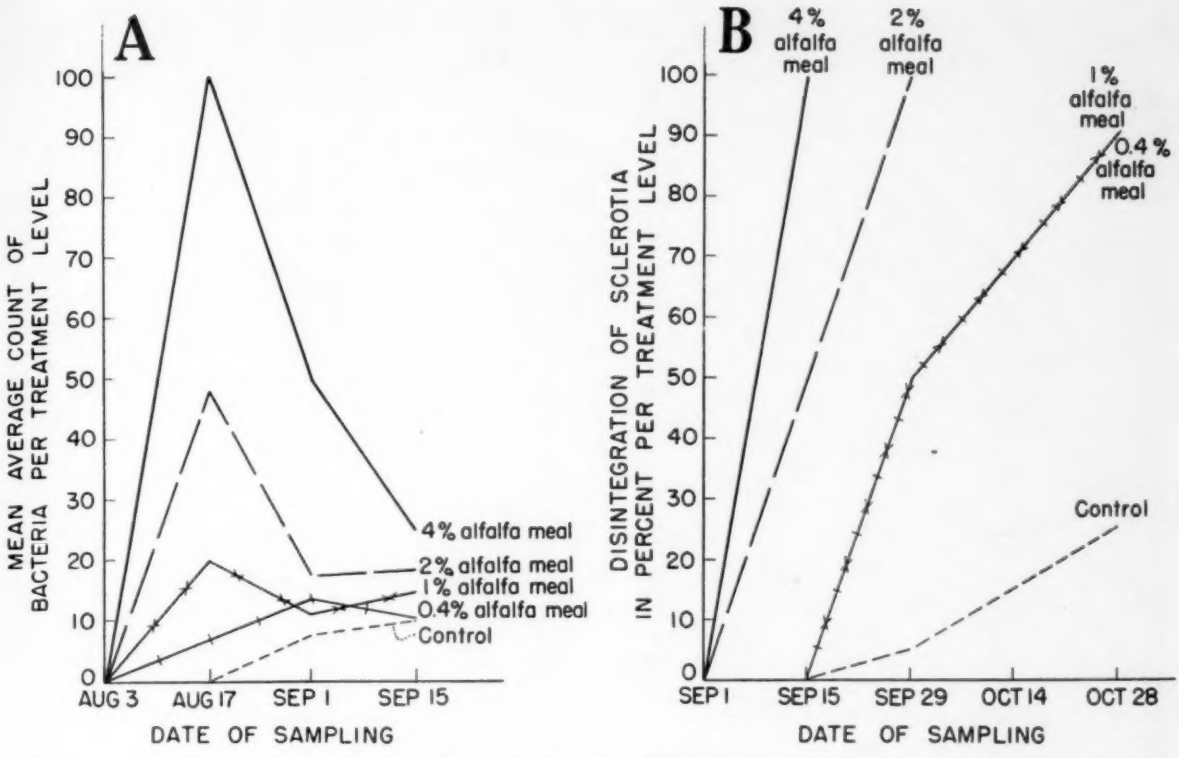

FIG. 2. A) Bacterial colony counts on potato dextrose agar plates at 10^{-6} dilution of 4 treatment levels of alfalfa meal—Lufkin soil mixtures; B) Percentage of disintegration of sclerotia in samples taken from the soil-meal mixtures.

Southern blight is prevalent in this soil, and peanuts, tomatoes, and other susceptable crops grown on this soil are usually attacked by the organism in late spring and early fall.

Fifteen 6-in. clay pots were filled with 3 kg. of alfalfa meal plus Lufkin fine sandy loam soil. Three pots were used for each treatment consisting of varying levels of alfalfa meal, 0.0, 0.4, 1, 2, and 4 per cent by weight. Approximately 100 ml. of 98 per cent viable sclerotia obtained from oat cultures were incorporated into each pot, which was then covered with a clay lid and watered with distilled water to keep the soil moist during the experiment.

The temperature of each pot was taken at bi-weekly intervals and essentially did not vary from the external day and night temperatures which averaged 36° C. and 28° C. for the course of the soil plate count experiment. Temperatures after September 15 fell slowly until October 28, when 31° C. day and 22° C. night temperatures were recorded. pH determinations of the alfalfa meal-soil mixtures 2 weeks after initial watering were: 5.6 for the control, 5.0 for the 0.4 per cent level, 4.5 for the 1.0 per cent level, and 4.0 for both the 2.0 and 4.0 per cent levels. These pH values were maintained with only slight variation for the balance of the experiment.

To measure the microbiotic activity in the soils, bi-weekly standard soil plate counts at 10^{-6} dilution were made with Difco nutrient agar as the substrate. Bacterial colony counts were recorded in this manner for 30 days.

Samples of sclerotia for germination studies were taken at bi-weekly intervals commencing 4 weeks after initial watering on August 3. The sclerotia were germinated by placing on moist filter paper in Petri dishes and incubating at 32° C. for 48 hours.

Three months after the beginning of the experiment the pots were planted to peanuts. After 10 days the seedlings were removed and the number bearing lesions recorded.

In the antagonism studies, 1 gm. of soil diluted to 10^{-4}, 10^{-5}, and 10^{-6}, respectively, was placed in separate Petri dishes and covered with warm potato-dextrose agar. Microorganisms were allowed to develop for 24 hours. The plates were inoculated with *S. rolfsii* by means of 1 infected oat grain each and incubated 5 days at room temperature, after which growth was recorded.

The microflora population increased with increasing levels of alfalfa meal during the first 2 weeks after initial watering, but rapidly fell off thereafter (Fig. 2A). The increased population of bacteria in the soil indicates that the presence of organic matter (alfalfa meal) materially increased the microfloral population and that the 2 probable limiting factors for their increase in Lufkin soil were carbon and nitrogen.

Evidence that the destruction of sclerotia and highest level of microflora activity were directly corre-

TABLE 1.—*Residual infectivity of S. rolfsii at various levels of alfalfa meal-soil mixtures planted to peanuts, November 7, 1951*

Percentage of Alfalfa Meal	Total Planted	Without Lesions	Failed to Germinate
4	24	22	0
2	24	11	4
1	24	2	13
0.4	24	13	0
0.0	24	3	3

lated is presented in Figure 2B. However it is evident that destruction of sclerotia in all cases followed the time of highest microbial activity by several weeks. The disappearance of the mycelial mantle preceded destruction of the sclerotia by 25 days at the 4 per cent level and 40 days at the 2 per cent level.

From data in Table 1, it is evident that mycelial fragments play a major role in the parasitic activity of *S. rolfsii* in the absence of viable sclerotia. This conclusion is not contrary to the findings of Epps et al. (2) that viability of mycelia fell off rapidly when stored at 50 per cent relative humidity and room temperature. The seeds of many plant species often fail to germinate in the soil because of low oxygen tension, under conditions where temperature and moisture are not limiting. When the soil is disturbed so as to relieve the oxygen stress, the seeds germinate readily. Such apparently was the case with the mycelial fragments in this experiment.

From data in Figure 3, it is evident that an inherent microfloral antagonism toward *S. rolfsii* existed in the Lufkin soil. The effect of this antagonism increased with the addition of alfalfa meal to the soil. At 10^{-4} dilution, the control (no added alfalfa meal) showed a distinct inhibition of the growth of *S. rolfsii* with little inhibition occurring at 10^{-5} and no inhibition at 10^{-6}. Whereas with the addition of 4 per cent alfalfa meal, inhibition occurred at all dilutions.

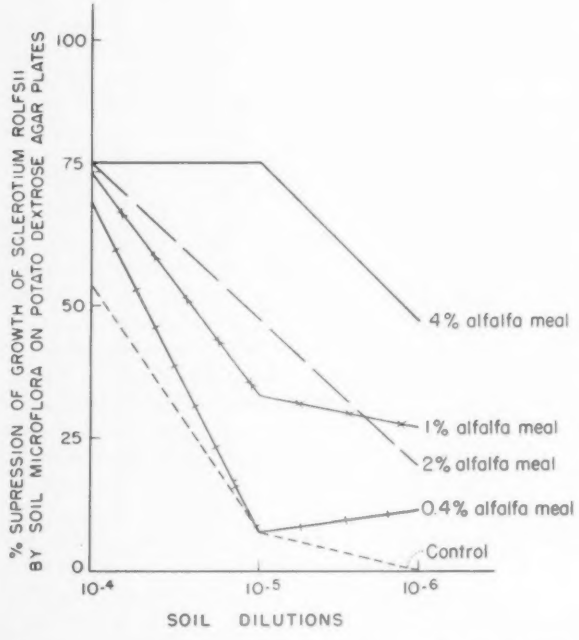

FIG. 3. Suppression of growth of *S. rolfsii* by a competing soil microflora at 3 dilutions of the soil-meal mixtures.

Figure 4 shows the antagonistic effect of the soil microflora toward *S. rolfsii*.

DISCUSSIONS AND CONCLUSIONS.—Lufkin soil has the capacity for a microfloral antagonism toward *S. rolfsii*, but the antagonism was not manifested in the destruction of sclerotia and mycelia unless the microflora were increased by the addition of organic matter. It is also evident from the present study that 4 per cent alfalfa meal by weight in a soil mixture satisfied the 2 limiting factors, carbon and nitrogen, as evidenced by the disappearance of the sclerotia and a marked reduction in the disease incidence in the peanut plants.

This suggests that the negative results reported by Langley (7) were possibly attributable to the inherently low organic matter (carbon content) of the soil of this area in Texas.

The ratio of soil carbon to nitrogen may also explain the reduction of southern blight noted by Leach and Davey (8) after adding nitrogenous fertilizers. The present study, in which the nutrition of *S. rolfsii* was investigated under controlled conditions, showed that the organism tolerates rather high levels of either ammonium or nitrate salts. The higher levels employed were greatly in excess of the usual nitrogenous fertilizer application. Possibly, the applications of nitrogenous fertilizers in Leach and Davey's work satisfied the nitrogen requirement of the soil microflora in a soil in which the carbon source was not the limiting factor.

Since it was shown that *S. rolfsii* had a high degree of tolerance to calcium, the practice of liming to reduce southern blight appears to be primarily an effect of raising the pH of the soil to 7.5 or higher. This agrees with the conclusion of Higgins (5) who attributed the reduction of the disease in sweet potato slip-beds to liming the beds to a point at which the pH was higher than could be tolerated by the organism. If the amount of lime used by Leach and Davey (9) was not sufficient to raise the pH to 7.5 or higher, then this would also account for their conclusion that liming had little effect in reducing the disease.

Little is known of the concentrations or the effect of the nitrite ion in the soil. Fraps and Sterges (4) estimated that nitrous nitrogen occurred at the rate of 0.04–0.06 p.p.m. in 12 field soils in Texas. Following applications of $(NH_4)_2SO_4$ and $CaCO_3$, the value in some basic soils ran in excess of 300 p.p.m. In all cases, the highest accumulation occurred when the soil was at 50 per cent water saturation. Waksman (11) found that nitrification stopped at pH values in the range of 4.4–4.8. Caster et al (1) reported considerable nitrite formation in Arizona soils only when pH values rose above 7.7.

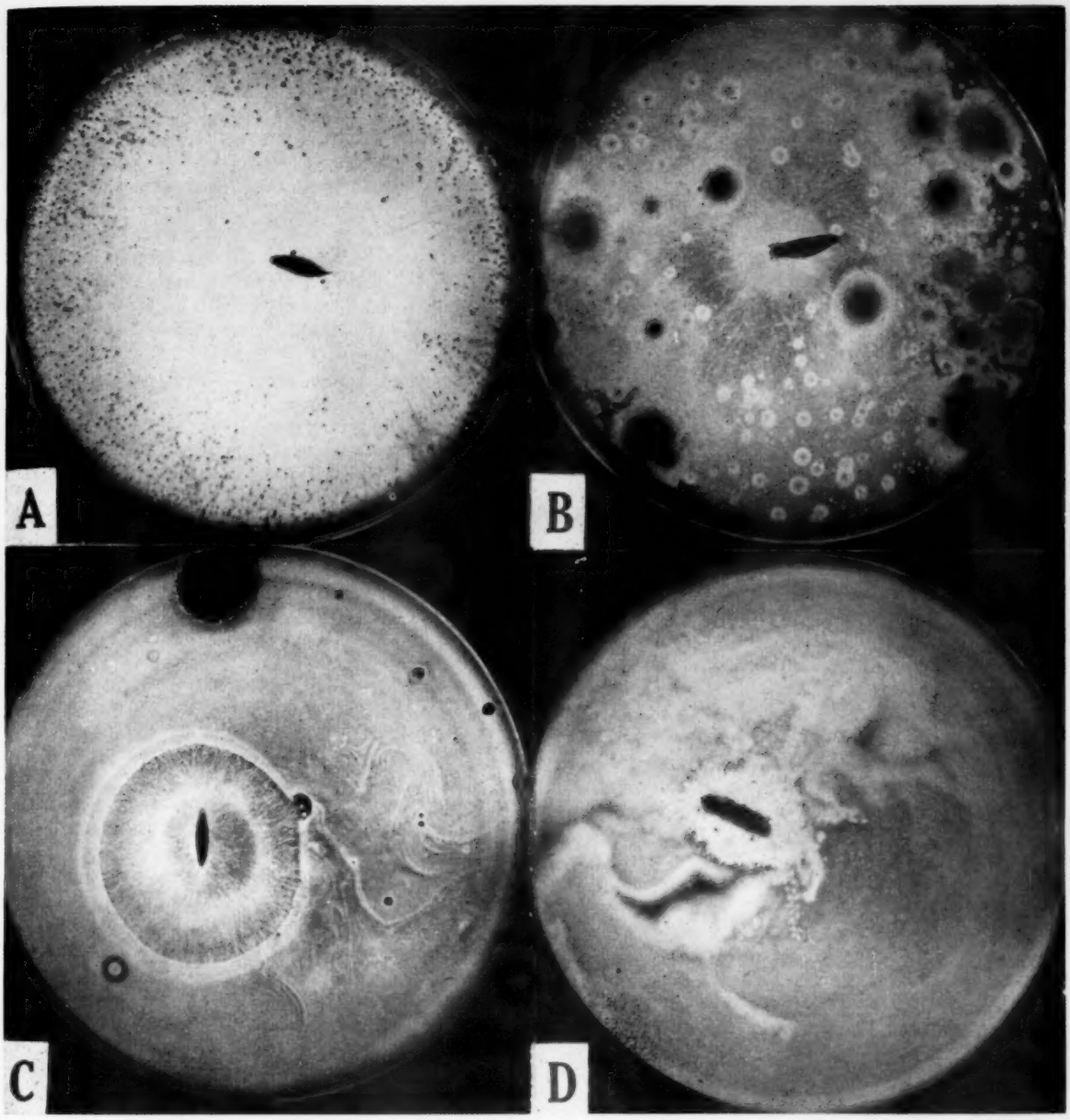

FIG. 4. Antagonism toward *S. rolfsii* as exhibited on potato-dextrose agar plates at 10^{-6} dilution of Lufkin soil; A) Normal uninhibited growth from the control; B) Antagonism exhibited by microfloral colonies from 4 per cent alfalfa meal; C) Mutual antagonism between an unknown bacterium and *S. rolfsii;* D) Suppression of growth by an unknown bacterium.

From the data of the present study it was found that nitrites became toxic to *S. rolfsii* at concentrations above 20 p.p.m., with complete suppression of growth at 400 p.p.m. However, in view of the reported basic pH requirement for nitrite accumulation, nitrites evidentally do not play a prominent role in the antagonistic effect noted toward *S. rolfsii* particularly in an acid soil such as Lufkin fine sandy loam.

Attempts to isolate specific antagonizing organisms were not made. It is believed that only a part of the total antagonism occurring in the soil was actually developed by the potato-dextrose agar culture method since many soil organisms do not grow on this medium. The data showed that a time lag existed between the occurrence of sclerotial disintegration and the time of highest microfloral population in any one level of alfalfa meal-soil mixture. Higgins (5) noted that *S. rolfsii* spread rapidly when sweet potato slip-beds were fertilized with cotton seed which is notably high in nitrogen and carbon sources. Thus it is possible that the organisms which are most active in the initial stages of alfalfa meal and cotton seed decomposition

may not necessarily be the same organisms which produce the antagonistic effect toward *S. rolfsii*.

The balance in the antagonism depends primarily upon the nutritional level of the soil. Under conditions of low soil nitrogen and low organic matter *S. rolfsii* may dominate in the microfloral population through its ability to exist parasitically whereas under conditions of high soil nitrogen and high organic matter the microflora antagonistic to *S. rolfsii* flourish. It is evident from the data obtained that the microorganism or organisms antagonistic to *S. rolfsii* are in the ascendency when the soil contains rapidly decomposing organic material, such as alfalfa meal, rich in both available carbon and nitrogen substrates.

SUMMARY

Sclerotium rolfsii was cultured under controlled conditions on synthetic liquid media. It was found that the organism tolerated extremely high levels of either ammonium or nitrate salts before adverse effects on growth were noted. The nitrogen salts investigated and the maximum concentration for good growth are: ammonium nitrate 16,000 p.p.m., sodium nitrate 16,000 p.p.m., and ammonium sulfate 36,000 p.p.m.

The inhibition of growth by calcium was determined. *S. rolfsii* tolerates 3000 p.p.m. before a decrease in growth is noted.

Nitrite was found to be toxic at levels in excess of 20 p.p.m., with complete suppression of growth occurring at 400 p.p.m.

Alfalfa meal was added to Lufkin sandy loam soil so that the final mixtures were 0.0, 0.4, 1, 2, and 4 per cent alfalfa meal by weight. Equal amounts of sclerotia were added to all levels of soil-meal mixtures and sclerotia disintegration studied. Total disintegration of the sclerotia occurred in 6 and 8 weeks, respectively, at the 4 and 2 per cent soil-meal levels, whereas only 90 per cent disintegration occurred in the 1 and 0.4 per cent soil-meal levels at the end of a 12-week period. In the control (Lufkin soil without added alfalfa meal) only 25 per cent disintegration occurred in 12 weeks.

Even though viable sclerotia were absent in the higher soil-meal mixtures, it was demonstrated that peanut seedlings germinated in the soil-meal mixtures became infected with southern blight. This infection was undoubtedly due to viable mycelia remaining in the soil.

A soil microfloral antagonism toward *S. rolfsii* was demonstrated in Lufkin soil, and the factors involved are discussed.

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INHERITANCE OF RESISTANCE IN BARLEY TO PHYSIOLOGIC RACES OF USTILAGO NUDA [1]

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The loose smut disease of barley has frequently reached troublesome proportions in New York State. Losses have been relatively light, except in a few instances, but the disease persists as a constant threat to barley production in the area. Yield reductions caused by this disease are approximately equivalent to the percentage of infected spikes appearing in the crop (9), and may account in part for the downward trend in the barley acreage of the state during the past decade.

The loose smut caused by *Ustilago nuda* (Jens.) Rostr., is by far the most abundant and the most difficult to control of the 3 smuts (*U. nuda* (Jens.) Rostr., *U. nigra* Tapke, *U. hordei* (Pers.) Lagerh.) that affect barley. *U. nuda* was identified in 90 per cent of the smutted barley spikes collected at random from farmers' fields in New York State during 1950 and 1951 (1).

With the smut-susceptible varieties presently available to the farmer, considerable difficulties are encountered in production and maintenance of smut-free seed. The hot water treatment effectively controls smut in infected seed, but it is difficult to use, expensive, and reduces seed germination. Furthermore, because of the danger of reinfestation from windborne spores of the fungus, smut-free seed can only be produced in isolated fields. This means that, at the present time, resistant varieties offer the most satisfactory and economical means of loose smut control.

A number of inheritance studies have been published (summary by Smith (10), (4) and (8)), but application of the results reported may be limited because of the racial specialization of the pathogen. The present study was designed to gain information concerning the inheritance of resistance to the known races of *U. nuda*.

MATERIALS AND METHODS.—Parental varieties used in the barley crosses included the U. nuda immune or highly resistant varieties Jet C.I. 967 and Valki C.I. 5478, and the highly susceptible variety Odessa C.I. 934[3]. Data from the present study are in Table 1.

Inoculum of tentatively-determined physiologic races was obtained from Dr. V. F. Tapke, U.S.D.A., Beltsville, Maryland. Inoculum was increased 1–2 times on the susceptible variety Odessa. The mature smutted spikes containing each race were kept in double glassine bags, closed with paper clips. The glassine bags were further separated by means of paper towels and stored in a refrigerator at 3–5° C.

TABLE 1.—*Reaction of parental varieties to tentative physiologic races of Ustilago nuda (Jens.) Rostr. Summarized from check rows in 1951 progeny tests*

Race	Jet	Valki[c]	Odessa		
			Plants	Smut per cent	Infection Range per cent
1	0/465[d]	0/466[d]	475/620[d]	76.6	30-100
2	0/150[a]	0/150[a]	292/310	94.3	30-100
3	0/133	0/65	117/126	92.9	70-100
4	0/134	0/55	255/267	95.5	30-100
5	0/73	0/55	143/169	84.6	40-100
6	0/466	0/325	401/436	92.0	60-100
7	0/306	0/217	621/773	80.3	35-100
8	0/100[a]	0/100[a]	179/216	82.9	40-100
Mixture[b]	0/100[a]	0/100[a]	206/208	99.0	90-100

[a] Estimated number tested, actual number not recorded.
[b] Equal mixture of 8 races.
[c] Valki has recently shown susceptibility to an apparently new race of smut. Unpublished results of Dr. R. G. Shands, University of Wisconsin, confirmed by Dr. V. F. Tapke, U.S.D.A., Beltsville, Maryland.
[d] Numerator, number of plants infected; denominator, number heading.

The spore-suspension method of inoculation was followed using 10 cc. hypodermic syringes with no. 20 needles 1 inch in length. The method and apparatus were suggested to the author by Dr. V. F. Tapke as satisfactory for maintenance of race purity. Glassware and equipment were autoclaved at 15 lbs. pressure for 15–20 minutes before each use. Spore suspensions were made up fresh each time. The most satisfactory spore concentration seemed to be approximately ½ gm. of spores in a liter of distilled water. Samples of chlamydospores from each smutted head used as inoculum were germinated at frequent intervals to ascertain species purity and percentage of germination.

Barley spikes were inoculated at flowering of the median florets and usually 1 day following. With 6-rowed varieties either central florets only were used, or lateral florets were inoculated 1 day following inoculation of the central florets.

Inoculations were made in the greenhouse and in the field. Inoculated seeds from F_1 plants were germinated in the laboratory to reduce mortality. The seedlings were transferred to pots in the greenhouse, or to rows in a small isolated field. Noninfected F_2 plants in each of the 3 crosses were reinoculated, for the most part, and their progenies grown in replicated,

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[3] Varietal reaction was based on unpublished information from Dr. V. F. Tapke.

randomized rows along with numerous rows of parental varieties similarly treated. Inoculated seeds for the F_3 tests were dusted with Arasan (tetramethylthiuram-disulfide), to control surface-borne fungi, and sown in the field.

Plants were classified as either healthy or smutted. Those with a single smutted tiller were classified as smutted.

Inheritance data were obtained from separate populations of the following crosses and their reciprocals: Jet × Odessa, Valki × Odessa, and Jet × Valki. Each population was inoculated with races 1, 6, and 7 of *U. nuda*. Similar but more limited studies were conducted with races 2, 3, 4, 5, 8 and a mixture of the 8 race collections. Genetic interpretations of the number of genes controlling resistance to the races studied were based on F_2 segregations corrected by progeny tests of reinoculated smut-free F_2 plants. Over 81 per cent of the entire population of smut-free F_2 plants obtained were tested for susceptibility. Since the crosses and reciprocals reacted identically, the data obtained from them were combined in the analysis for each race.

Segregations of morphological characters in the cross Jet × Odessa inoculated with races 1, 6, and 7 were recorded. Classification was made on F_2 plants and generally verified in F_3. Data from the resistant and segregating lines were summarized to determine whether or not any of the morphological characters were associated with loose smut resistance. The procedure used did not permit classification of morphological characters on susceptible F_2 plants.

EXPERIMENTAL RESULTS.—*Relationship of viability of inoculated seed to infection by U. nuda.*—A positive correlation between loose smut susceptibility and lowered viability of inoculated seeds would influence the results of this study. Data were colleced on numbers of inoculated seeds of parental varieties, and crosses in F_2 and F_3 generations which did not produce plants. These data were summarized from the studies with races 1 and 6, 2 of the largest populations inoculated, and in which a number of susceptible lines were sorted out in the F_3 tests.

Both Odessa and Valki had a lower percentage survival under artificial inoculation in the F_2 tests than did the variety Jet, although lower survival was not apparent for Odessa in the F_3 tests. Furthermore, the heritability of the cause of higher survival ability as a dominant trait was evident in the viability of seed of the crosses involving Jet in both F_2 and F_3 tests (Table 2). Noninoculated seed of parents and crosses were grown and showed uniformly high viability (about 85 per cent or over in both years). A chi-square test revealed no indication of an association of lowered viability with susceptibility of F_3 lines of all 3 crosses inoculated with races 1 and 6. Results suggest that some factor other than infection by *U. nuda* is responsible for lowered viability.

F_2 Results.—Infection of the embryo by *Ustilago nuda* occurs in the barley spike shortly after flowering

TABLE 2.—*Viability[a] of seeds of parental varieties and crosses inoculated with races of U. nuda*

Parent or Cross	Race 1		Race 6	
	No. of Seeds Tested	Percentage Survival	No. of Seeds Tested	Percentage Survival
Jet (F_2 tests)[b]	75	58.7	100	85.0
Valki (F_2 tests)	92	41.3	65	67.7
Odessa (F_2 tests)	125	48.8	116	57.8
Odessa × Jet (F_2)	227	79.7	206	79.1
Odessa × Valki (F_2)	221	67.4	212	51.4
Jet × Valki (F_2)	226	76.1	207	79.2
Jet (F_3 tests)	465	77.9	647	70.8
Valki (F_3 tests)	466	67.8	530	61.3
Odessa (F_3 tests)	602	69.0	711	69.2
Odessa × Jet (F_3)	3508	67.1	3640	71.3
Odessa × Valki (F_3)	3409	60.1	2395	56.3
Jet × Valki (F_3)	3753	67.8	3415	66.0

[a] Based on total number of plants obtained.
[b] F_2 were grown both in the field and in the greenhouse, whereas all F_3 were grown in the field.

time and the infecting smut hyphae, in certain instances, must pass through genetically resistant ovary tissue before reaching a susceptible embryo (2) (7). The appearance of infected plants in the progeny of an F_1 hybrid of resistant × susceptible varieties, resistance being dominant, suggests that resistance is determined by the genetic constitution of the F_2 embryo. Some infected plants were obtained in the F_2 of both resistant × susceptible and susceptible × resistant barley crosses inoculated with physiologic forms of *U. nuda* (Table 3), presenting evidence that resistance was governed largely by the inherent nature of the embryo and not by the genetic constitution of the maternal tissue surrounding it. In no case, however, was the expected number of smutted plants obtained in the F_2 tests. Therefore, since there is no reason to suspect differences in moisture relations or inoculum it is probable that the resistant maternal tissue caused some inhibition of infection.

F_3 Results.—Results obtained in the F_2 generation and in further tests of smut-free F_2 plants of the crosses inoculated with the particular *U. nuda* races indicate that the Valki and Jet types of resistance are dominant (Tables 3 and 4).

Data obtained from populations of the 3 crosses inoculated with each of the 8 races of *U. nuda* and with a mixture of these races is in Table 4. A wide range in infection percentages was obtained among F_3 progenies, as expected, since some plants escaped infection, and population size among progenies was variable (average 14–21 plants depending on the race of smut used).

In combining data for the factorial analyses, it was obvious that only 2 classes could be distinguished with reasonable certainty, i.e., resistant and segregating vs. susceptible because "escapes" were found to occur in segregating progenies as discussed before. Smutted F_2 plants were arbitrarily considered as 100 per cent susceptible in F_3, and were added to the populations verified by the F_3 tests.

TABLE 3.—*Infection obtained in F_2 and summary of inheritance ratios obtained from crosses involving Jet, Valki, and Odessa inoculated with physiologic forms of U. nuda*

Cross	Race	Total Plants Obtained in F_2	Percentage of Infection in F_2 only	Probable Number of factor Pairs Involved	Theoretical Ratio[b]	Probability Greater Than
Jet × Valki	1	184	0.0	2	15:1	.50
Odessa × Valki	1	156	3.3	1	3:1	.30
Odessa × Jet	1	167	18.4	1	3:1	.50
Jet × Valki	2	74	0.0	2 (4)[c]	15:1	.30
Odessa × Valki	2	66	13.6	2	9:7	.20
Odessa × Jet	2	66	15.2	2	9:7	.80
Jet × Valki	3	69	0.0			
Odessa × Valki	3	73	15.0	2	9:7	.20
Odessa × Jet	3	83	1.2	2	15:1	.30
Jet × Valki	4	95	0.0			
Odessa × Valki	4	56	0.0	2	15:1	.70
Odessa × Jet	4	79	0.0	2	15:1	.10
Jet × Valki	5	85	0.0	4[d], 2	247:9[d], 15:1[e]	.20, .05
Odessa × Valki	5	86	1.2	2	13:3[d], 3:1[e]	.70, .10
Odessa × Jet	5	88	5.7	2	13:3[d]	.30
Jet × Valki	6	170	0.6	3	57:7	.20
Odessa × Valki	6	122	6.6	2	9:7	.50
Odessa × Jet	6	174	13.8	1	3:1	.50
Jet × Valki	7	90	0.0	4[d]	249:7	.10
Odessa × Valki	7	73	32.9	2	9:7	.70
Odessa × Jet	7	148	4.1	2	15:1	.30
Jet × Valki	8	78	0.0			
Odessa × Valki	8	80	0.0	2	15:1[d], 13:3[e]	.10, .10
Odessa × Jet	8	82	2.4	2	15:1	.80
Jet × Valki	Mix[a]	55	0.0			
Odessa × Valki	Mix[a]	48	25.0		1:3	.30
Odessa × Jet	Mix[a]	53	5.7		3:1	.80

[a] Mixture of 8 races in equal proportions.
[b] Based on combined F_2 and F_3 data.
[c] Linkage apparently involved.
[d] Suggested but not enough data for support.
[e] Data fit both ratios, probability for each given.

Class intervals in the frequency distribution were placed at 5 per cent infection since it appeared to be the best way to determine the point at which a break between segregating and susceptible progenies occurred. The separation point between resistant-segregating and susceptible progenies in the F_3 tests appeared to be at 50 per cent infection, except for populations inoculated with races 4 and 5 where this point was at 45 per cent (Table 4). The influence of maternal tissue in inhibiting infection in segregating progenies was evident with the F_3 as with the F_2 data, since the number of segregating progenies was far below that expected. Therefore, it was assumed that part of the segregating progenies failed to show smut and were placed among the resistant or immune class.

Inheritance ratios and factorial analyses.—The F_3 progeny tests not only permitted a correction of the F_2 classification by sorting out the "escaped" susceptibles, but also provided a factor for use in adjusting the results from those portions of the populations where reinoculation for F_3 progeny tests was not possible. This factor, obtained from the percentages of "escaped" susceptibles found among the healthy F_2 plants re-inoculated and progeny tested, was applied to the number of healthy F_2 plants of the same cross not classified by F_3 data. The expected number of susceptible and resistant plants were thus determined. The adjustments should tend to give more accurate ratios, but in all cases where population numbers studied were sufficient to indicate a fit to only 1 possible segregation ratio, the changes were slight and unimportant.

Detailed analyses will be presented only for data obtained on segregations for resistance to races 1, 6, and 7, since larger populations were studied. Results obtained with the other races will be discussed briefly.

Race 1.—From the cross Odessa × Jet (susceptible × resistant), 26 smutted plants were obtained in the F_2 generation (Table 4). An additional 15 plants were found to have escaped infection in F_2 but produced progenies over 50 per cent infected in F_3. A total of 55 progenies from reinoculated healthy F_2 plants were smut free, and 57 progenies were segregating for susceptibility. Odessa × Valki (susceptible × resistant) produced 55 smut free, 43 segregating, and 32 susceptible progenies in F_3. Five plants were smutted in F_2. The 159 progenies of Jet × Valki (resistant × resistant) included 117 that were smut-free, 34 that were segregating for smut reaction, and 8 that were smut-susceptible.

Table 4

Summary of F_2 and F_3 data recorded in studies involving crosses of three barley varieties inoculated with 8 races and a bulk of the 8 races of U. nuda (Jens.) Rostr.

Cross	Race	Infection Percentage Classes: Resistant and Segregating — Number of Plant Progenies: 0	.1-5	5.1-10	10.1-15	15.1-20	20.1-25	25.1-30	30.1-35	35.1-40	40.1-45	45.1-50	50.1-55	55.1-60	60.1-65	Susceptible: 65.1-70	70.1-75	75.1-80	80.1-85	85.1-90	90.1-95	95.1-100	F_2 Smutted	Odessa Check Total	Total F_2 Verified for Susc. or Res.
Jet x Valki	1	117	3	10	4	3	4	2	1	3	2	2	0	0	0	3	2	1	0	1	0	1			159
Odessa x Valki	1	55	2	10	7	8	4	5	0	0	4	1	0	3	1	2	4	4	2	5	4	7	5		133
Odessa x Jet	1	55	4	7	11	10	6	7	2	7	1	2	2	1	5	1	1	1	1	1	1	1	26		153
Odessa Check								1	1	4	1	2		1	1	2	4	8	2	12	1	36		76	
Jet x Valki	2	51*				7*															6*		0		64
Odessa x Valki	2	15*				12*															20*		9		56
Odessa x Jet	2	14*				17*															13*		10		54
Odessa Check								1				1					1	1		2	1	28		35	
Jet x Valki	3	45	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		46
Odessa x Valki	3	11	0	4	1	3	2	4	0	1	0	0	1	0	0	0	0	1	0	0	1	0	11		40
Odessa x Jet	3	47	1	3	4	1	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1		60
Odessa Check																1	3	2		1	1	18		26	
Jet x Valki	4	86	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		86
Odessa x Valki	4	28	0	5	2	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0		39
Odessa x Jet	4	51	0	2	2	3	2	0	0	0	0	1	0	1	0	1	1	1	1	1	0	0	0		67
Odessa Check								1								3		1		2	2	8		17	
Jet x Valki	5	72	1	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0		76
Odessa x Valki	5	43	0	4	2	3	1	0	0	0	0	1	0	2	0	1	0	1	0	3	2	0	1		64
Odessa x Jet	5	38	1	7	3	9	2	0	0	0	0	2	0	0	0	1	0	1	1	0	0	1	5		71
Odessa Check										1			2			2		1		2	1	5		14	
Jet x Valki	6	95	3	7	6	4	1	6	1	2	2	0	0	3	0	0	1	0	0	1	2	3	1		138
Odessa x Valki	6	21	3	5	3	9	5	3	0	4	2	2	1	2	0	2	1	1	3	3	4	13	8		95
Odessa x Jet	6	51	1	7	11	10	20	5	2	3	7	2	1	1	2	2	0	0	2	2	4	5	24		162
Odessa Check														2		4	3	3	3	4	3	32		51	
Jet x Valki	7	75	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		76
Odessa x Valki	7	8	0	3	2	3	3	7	2	2	2	2	1	0	0	0	0	0	0	1	1	2	24		63
Odessa x Jet	7	80	7	9	6	5	1	2	4	1	0	2	0	1	0	0	0	1	0	1	0	2	5		127
Odessa Check									1	1	1			4		10	2	4	2	8	2	46		81	
Jet x Valki	8	65*																					0		65
Odessa x Valki	8	50*				8*															7*		0		65
Odessa x Jet	8	53*				15*															3*		2		73
Odessa Check										2		1		1			5	1	5	1		13		29	
Jet x Valki	Mix	41*				2*															13*		0		43
Odessa x Valki	Mix	8*				3*															8*		12		36
Odessa x Jet	Mix	18*				13*																	3		42
Odessa Check																				2		30		32	

*Data on percent infection were not recorded.

Data from Odessa × Valki, arranged in 2 classes, fits a 3:1 theoretical ratio with a probability greater than .30, whereas the segregation obtained from Odessa × Jet fits this same ratio with a probability greater than .50. That from Jet × Valki fits a 15:1 expected ratio with a probability greater than .50, and not only confirms the results obtained with the other 2 crosses with regard to the dominance of resistance to race 1 and its simple Mendelian inheritance but also shows that the Valki and Jet genes are different and independent (Table 3).

Race 6.—There were 51 resistant, 68 segregating and 19 susceptible progenies of Odessa × Jet grown from inoculated healthy F_2 plants. An additional 24 smutted plants were sorted out in the F_2 generation. In this cross 3 inoculated F_2 plants produced some smutted and some normal tillers. On these plants, noninfected tillers were reinoculated and grown in the F_3 tests. They proved to be completely susceptible, indicating that they had been correctly classified as susceptible in the F_2 generation. With the cross Odessa × Valki, only 8 smutted plants appeared in the F_2 generation. However, among the healthy F_2 plants reinoculated, 21 produced smut-free, 36 segregating and 30 susceptible progenies. One smutted plant of Jet × Valki occurred in the F_2 generation, and 10 additional plants proved susceptible in the F_3 tests, whereas 95 produced smut-free and 32 produced segregating progenies.

The Chi-square test for goodness of fit of the Odessa × Jet segregation to a 3:1 ratio gave a probability of greater than .50. An equally close fit but to a 9:7 theoretical ratio was obtained with Odessa × Valki. The observed segregation of Jet × Valki for resistance and susceptibility to race 6 approximated a 57:7 calculated ratio, and not only supports the conclusions derived from data provided by the other 2 crosses, but also shows that the Jet and Valki types of resistance are unrelated.

Race 7.—Five smutted plants occurred in the F_2 generation of Odessa × Jet inoculated with race 7, and among a total of 122 healthy plants tested in F_3, only 5 produced susceptible progenies whereas 37 progenies were classified as segregating and 80 were smut-free. There were 24 smutted F_2 plants of Odessa × Valki, and an additional 5 were sorted out in the F_3 tests. Twenty-six progenies proved heterozygous for susceptibility and only 8 were smut-free. Smut susceptibility was absent in the F_2 generation of Jet × Valki inoculated with this race. Furthermore, the only susceptibility found in this latter cross was in the F_3 generation tests in which a single heterozygous progeny produced 5 per cent smut. The number of progenies tested was insufficient to determine the inheritance pattern.

The ratio of 117 resistant to 10 susceptible plants obtained in Odessa × Jet closely approximated a 15:1 theoretical ratio, indicating the presence of 2 equally effective dominant resistance factors in Jet. The Chi-square test of the 34:29 segregation in Odessa × Valki for resistance to race 7 for goodness of fit to a 9:7 theoretical ratio gave a probability greater than .70.

The population of Jet × Valki was inadequate for the determination of linkage of the Jet and Valki resistance factors. However, the segregation obtained fits a 249:7 ratio with a probability greater than .10 and it may be assumed from the fact that the resistance types are different, that the genetic basis for resistance in the 2 varieties is not associated. The lack of susceptibility among the 76 plants of Jet × Valki tested is also supporting evidence for the correctness of the ratios obtained with the other 2 crosses.

Data obtained in studies involving races 2, 3, 4, 5, 8 and a race mixture have been summarized (Table 4). The populations studied are not considered large enough for a critical analysis, and therefore the results will not be presented in detail here. Although the results were not conclusive, the ratios obtained indicate certain inheritance patterns which should be recorded (Table 3).

Association of characters.—In 1 cross involving resistance vs. susceptibility, the parental varieties (Odessa and Jet) differed by 4 morphological characters known to be identified with particular linkage groups.

All the characters studied have been found to be independently inherited. A summary of studies involving these and other characters has been compiled recently by Smith (10). Therefore, individual references will not be cited.

The variety Odessa has 6-rowed spikes (vv), a white lemma and pericarp (bb), hulled caryopsis (NN), and long-haired rachillas (SS). Jet, in contrast, has 2 rowed spikes (VV), a black lemma and pericarp (BB), naked caryopsis (nn), and short-haired rachillas (ss). The genes (noted by the symbols) conditioning the characters studied are located in linkage groups I, II, III and V.

Classification was made on F_2 plants and verified in F_3 lines. Only the homozygous resistant and heterozygous plants in studies with races 1, 6, and 7 were used. Evidence for linkage would be found were there any great discrepancy in any ratio within this resistant population. Segregations obtained for all the morphological characters fit monohybrid ratios in agreement with published results. There is no evidence for linkage of genes governing resistance to races 1, 6, and 7 of *U. nuda* with the morphological markers used.

Discussion.—Jet and Valki proved to be highly resistant or immune and Odessa highly susceptible to all 8 of the physiologic races of *U. nuda* used in these studies.

The contention of Oort (5) that inherent loose smut resistance or susceptibility and viability of artificially inoculated seeds are separate phenomena is supported by data presented in this paper. No association was found to exist between these 2 phenomena in populations from 3 crosses inoculated with races 1 and 6 of *U. nuda*. Inoculated seeds of parental varieties,

however, differed in viability. Moreover, these differences were reflected in the F_2 populations produced from inoculated seeds of the crosses. Noninoculated seeds of both parents and of crosses showed high viability. Results of others (6), (11), (3), (8), have not agreed with the indications noted above. The apparent discrepancy may be explained by the evidence that both smut resistance or susceptibility and the control of the cause of high or low viability of inoculated seeds are inherited qualities which, in certain cases, may be associated.

The inhibiting effect of resistant maternal tissues on infection of susceptible embryos was evident in results obtained with all crosses and all races. Relatively few infected plants were obtained in the F_2 generation of inoculated crosses, and likewise the expected numbers of segregating progenies failed to appear in the F_3 tests of healthy F_2 plants. Similar results have been reported by Livingston (3) and Schaller (8).

The genetics of resistance in Jet and Valki was found to differ depending on the race of *U. nuda* used. Resistance appeared to be dominant to susceptibility in all crosses.

The studies reported herein were begun using tentatively determined physiologic races of loose smut, *U. nuda* (Jens.) Rostr. Some changes in racial status have been indicated by Tapke's most recent results. His recent[4] tests indicate that races 2 and 5 are similar to race 1, races 3 and 7 are identical, and that races 6 and 8 are the same as race 4. However, the genetic evidence obtained in the foregoing studies is in agreement with the combination of only races 3 and 7, 4 and 8.

Race 1 resistance was found to be determined by single independent genes in Jet and Valki. Race 6 resistance, however, required 2 Valki genes, but only 1 Jet gene, the former being a type of loose smut resistance not previously reported in barley. Resistance to race 7 was found to involve 2 factors in each resistant variety. Although either of the Jet genes was sufficient to confer its high resistance, both Valki genes were required. When data from studies with race 3 and 7 were combined, results were the same.

Results of studies with races 2, 4, 5, 8 and a race mixture could not be definitely interpreted factorially because of the limited populations obtained. Nevertheless, the data indicated that several types of smut resistance, probably due to a number of genetic factors, are found in just 2 varieties. If races 4 and 8 are identical, as mentioned before, more may be derived from the data obtained by a combination of the populations.

The combined race 4 and race 8 segregation for the cross Odessa × Valki is 94 resistant to 10 susceptible progenies. Similarly, for Odessa × Jet the segregation is now 128 resistant to 12 susceptible. The cross of Jet × Valki yielded only resistant progenies from the 110 F_2 plants tested. The ratios obtained from each of the susceptible × resistant crosses fit a 15:1 theoretical ratio, sufficiently establishing that 2 independently effective race 4 resistance factors are possessed by each of the varieties, Jet and Valki. Since only a single susceptible progeny would be expected in an F_2 population of 256, assuming independence of factors, the observed population of the cross Jet × Valki was insufficient in size for interpretation, except that the results obtained with this latter cross do not disagree with the information obtained with the other 2 crosses.

One can only speculate on results obtained with the mixture of races. A probable explanation is that competition between races may account for results obtained when the mixture was used.

No evidence of linkage of morphological markers Bb, Vv, Nn, and Ss was detected with races 1, 6, and 7 in the cross Odessa × Jet.

Further work is needed to establish the interrelationships between the resistance factors present in Jet, since this appears to be the better resistance source of the 2 varieties used. It is suggested that symbols not be assigned to any of the resistance factors until their interrelationships can be learned.

[4] Unpublished 1952 results of Dr. V. F. Tapke, U.S.D.A., Beltsville, Maryland received by personal correspondence.

SUMMARY

Evidence was presented which demonstrated that resistance to different loose smut races may be inherited differently depending on the physiologic race of the pathogen and the variety of the host.

Resistance to race 1 of *U. nuda* was found to be determined by a single dominant gene present in each of the varieties Jet and Valki. Jet and Valki both possessed 2 genes for resistance to races 3 and 7. Both Valki genes were required to confer resistance whereas either of the Jet genes would suffice.

Resistance to races 4 and 8 was conditioned by either of 2 independent genes present in each of the resistant varieties.

Resistance to race 6 was inherited differently in the 2 varieties. Jet possessed a single resistance factor, but 2 factors in Valki were indicated. Both Valki factors were needed for resistance.

Results of studies with races 2 and 5 were inconclusive because of the limited populations observed.

Viability of seeds inoculated with races 1 and 6 of *U. nuda* was not associated with inherent smut resistance or susceptibility.

No evidence of linkage of race 1, 3, or 6 resistance genes present in Jet with 4 morphological characters was obtained.

No attempt was made to assign gene symbols to the resistance genes found since it is believed that further study is desirable in order to define more clearly their inter-relationships.

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METHYL BROMIDE FOR NURSERY SOIL FUMIGATION [1]

Donald E. Munnecke and John Ferguson

Gaseous methyl bromide is commonly used in southern California to control soil pathogens in ground beds, nursery containers, and greenhouse benches. It has previously been reported (4, 8) to be an effective soil fungicide, but in another test (6) it was ineffective *in vitro* against *Fusarium sambucinum* and *Corynebacterium sepedonicum*. The chemical is easy to use, acts rapidly, and has left no harmful residue in either our tests or commercial use in the Los Angeles area. Because it is relatively expensive, toxic to man, and must be confined to be effective, its use is limited to small areas except in the case of valuable crops. Its effectiveness under local conditions was therefore tested, and the results are here reported.

No tests were conducted with liquid methyl bromide, as used by others (5, 8), since it is unsatisfactory for use in mass production methods employed in California nurseries where the daily fumigation of 300–500 flats is common.

Materials and methods.—The effectiveness of methyl bromide as a soil fungicide was tested by fumigating artificially infested soil and soil containing pure cultures of fungi enclosed in plastic screen bags. The infested soil was prepared by inoculating steamed U. C. soil mixture (1) either with *Rhizoctonia solani* or *Fusarium oxysporum* f. *callistephi* and growing susceptible seedlings in it. The pure cultures consisted of washed mycelial mats of *Fusarium* spp., *Rhizoctonia solani*, *Pythium* spp., and *Sclerotinia homeocarpa* grown in potato dextrose broth; sclerotia of *Sclerotium delphinii* and *Botrytis cinerea*; *Phytophthora citrophthora* growing on sterilized alfalfa stems; and microsclerotia of *Verticillium albo-atrum* on sterilized tomato stems. In addition camellia roots naturally infested with *Phytophthora cinnamomi* were used. The pure culture inoculum was placed in small plastic screen bags and buried in soil in flats or at measured depths in bulk soil.

Several methods were used to fumigate soils with methyl bromide. Flats filled with a sandy loam and peat mixture (1) were loosely stacked on the ground in a criss-cross fashion and enclosed beneath a gasproof polyethylene (Visqueen) cover. The cover, which enclosed a volume of 25–100 cu. ft. was sealed by placing soil on the lower edges. Best volatilization of the gas under the cover was obtained by passing the gas through copper tubing immersed in hot water (160°F.) before releasing it beneath the cover. The flats were placed in 5 ft. vertical stacks in most tests. The possible settling of the heavy gas was tested by burying fungi in flats at various levels. In no case were there differences in kill of the fungi from the top to the bottom of the stack. After a treatment period of 24 hours followed by a 24 hour aeration period, the soil was used for planting.

The effectiveness of methyl bromide treatment was determined by recovery of the fungi in culture, or by survival of susceptible hosts planted in the infested soil. The buried inoculum was removed from the plastic screen bags, washed in sterile water, and plated on pea straw agar plates (3). In this way the inoculum was easily recovered from the soil, and since the low-nutrient media eliminated most of the contaminants, the effect of the treatment could be easily and rapidly determined. Flats of infested soil which were treated with methyl bromide were planted after suitable aeration periods. The non-treated soil infested with either *Rhizoctonia solani* or *Fusarium oxysporum* f. *callistephi* produced both pre-emergence and post-emergence damping-off of peppers and China aster, respectively.

Results.—The effect of fumigation with methyl bromide (4 lbs. per 100 cu. ft. of space beneath the cover) on a number of fungi is shown in Table 1. Occasionally, the large mycelial mats of *Fusarium oxysporum* f. *gladioli* or *Rhizoctonia solani* were

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TABLE 1.—*Effect of gaseous methyl bromide applied at the rate of 4 pounds per 100 cu. ft. of space for 24 hours on fungi in soil in flats*

Fungus tested	Type of inoculum	Percentage control[a]
Botrytis cinerea	Sclerotia	100
Fusarium oxysporum f. *gladioli*	Mycelium on sterile pea straw	100
do	Mycelium on corn meal media	100
do	Mycelial mats	94[b]
Fusarium oxysporum f. *callistephi*	Mycelial mats	100
do	Infested soil	100
Phytophthora cinnamomi	Infested camellia roots	100
Phytophthora citrophthora	Mycelium on sterile alfalfa straw	100
Pythium ultimum	Mycelial mats	100
Rhizoctonia solani	Mycelial mats	94[c]
do	Infested soil	100
Sclerotinia homeocarpa	Mycelial mats with sclerotia	100
Sclerotium delphinii	Sclerotia	100
Verticillium albo-atrum	Microsclerotia on sterile tomato stems	66[d]

[a] Based on recovery in culture, or of survival of susceptible seedlings planted in the soil.
[b] 1/15 viable in 1 experiment, 1/18 viable in another.
[c] Results of 4 experiments: 1/36, 0/22, 0/12, and 4/11 viable cultures.
[d] No growth in 2 experiments, slow growth in another.

viable after fumigation. These mycelial mats represented masses of inoculum far in excess of that normally found in infested soil, and concurrent treatment of soil heavily infested with *Rhizoctonia* resulted in 100 per cent control. The microsclerotia of *Verticillium albo-atrum* are more difficult to kill than the other test fungi; thus gaseous methyl bromide at the standard dosage may be unsatisfactory for control of Verticillium wilt. No concurrent fumigation of Verticillium-infested soil was made to determine the effect of the gas on the fungus in soil. China asters grew to maturity without appearance of wilt in soil which was infested with *Fusarium oxysporum* f. *callistephi* and then treated with methyl bromide. *Fusarium* was isolated from plants grown in non-treated, but not in treated soils.

Since it is often desirable to fumigate soil near growing plants, the effect of methyl bromide upon a number of potted plants adjacent to a fumigation area was determined. Flats occupying 25 cu. ft. of space were stacked in a closed, unshaded, 10 × 10 × 9 ft. greenhouse chamber, covered with a gas-tight, polyethylene (Visqueen) cover and treated with 1 lb. of gaseous methyl bromide. The following potted plants were held about 3 ft. away during the treatment and subsequent aeration: China aster, azalea, beet, *Cyclamen*, bean, *Dracaena*, tomato, pea, geranium, *Petunia*, *Dieffenbachia*, *Fuchsia*, *Nephthytis*, watermelon Peperomia, greenleaf Peperomia, *Philodendron hastatum*, and *P. cordatum*. The temperatures during the 24 hour treatment period ranged from 60–89°F. and for the following 24 hour aeration period they ranged from 61–101°F. The greenhouse ventilators were opened wide just prior to removal of the plastic cover and were left open during the aeration period. Although plants were observed for several months, no injury was noted on them as a result of being placed near the fumigation area.

Newhall (7) recently reported that liquid methyl bromide caused some injury to chrysanthemums in an adjacent untreated greenhouse bench and he quoted unpublished reports concerning injury to carnation, celery, and beet seedlings caused by the chemical. Unfortunately, these plants were not used in our experiments, nor was the liquid compound compared with the gaseous form of methyl bromide. Probably little damage to adjacent vegetation will occur with gaseous methyl bromide if the area is well ventilated and the plants are held at least 3 ft. from the treated area.

The length of the necessary soil aeration period was determined by planting seeds or transplants in soil treated with the gas. In 1 test China aster, bean, pea, and pepper seeds were planted in flats after a 3-hour aeration period. The peppers germinated poorly and were stunted, but the other seeds produced plants comparable to those grown in steam soil. In another test, stunting occurred only in soil aerated for periods less than 20 hours, and a few asters were killed in soil aerated for only 3 hours. As a result 24-hour aeration was used in subsequent experimental work with flats.

Since ground beds are often fumigated for disease control, it is important to know how deep methyl bromide will penetrate. Sclerotia of *Sclerotium delphinii* were enclosed in plastic screen bags and buried at depths of 1, 3, 6, 9, 12, 18, and 30 in. in a heavy clay loam (Yolo series). All of the sclerotia were killed, even at the 30-in. level, when 5 lbs. of methyl bromide were used per 100 sq. ft. of surface. Soil temperatures at the 2-in. level varied from 55°–65° F., but at the 30-in. level remained at 59° F. The exposure period was 48 hours. Sclerotia buried 6 in. in an adjacent untreated bed were all viable. In another test sclerotia of *Sclerotium delphinii*, mycelial mats of *Rhizoctonia solani*, and corn meal media containing *Fusarium oxysporum* f. *callistephi* mycelium were used as test organisms. The soil was a sandy loam devoid of organic matter, confined in a bin 6.5 × 5.5 × 4 ft. The application rate was 4 lbs. per 100 cu. ft. The temperature dropped to approximately 55° F. and it

rained after the cover was applied. Sclerotia of *Sclerotium delphinii* were killed at the 3, 6, 12, and 18-in. levels, but not at the 30- or 40-in. levels. The colonies developing from treated sclerotia at the 30-in. level were much smaller than those at the 40-in. level or the checks, indicating some effect of the gas at the 30-in. level. Also, growth of microsclerotia of *Verticillium albo-atrum* was greatly reduced in a like manner after treatment with methyl bromide in another experiment. The mycelium of *Rhizoctonia* was killed at 6 in. with some inhibition of growth at the 12-in. level, but was unaffected at lower depths.

During the winter months of 1951-52 many nurserymen in the Los Angeles-San Diego area successfully used methyl bromide to fumigate soils in flats for bedding plants. A survey and reports of farm advisors and nursery inspectors indicate that very satisfactory control of damping-off has been obtained by treatment with methyl bromide.

Although the recommended rate for control of soil fungi is 4 lbs. per 100 cu. ft., growers have sometimes successfully used lower dosages. To determine necessary dosage rates, Rhizoctonia and Fusarium-infested soils were fumigated at rates of 1, 2, 3, and 4 lbs. per 100 cu. ft. No control of either organism was obtained with less than 3 lbs. per 100 cu. ft. The 3-lb. rate was satisfactory for control of Rhizoctonia damping-off, but not for aster wilt. Chisholm and Koblitsky (2) found that the soil type and its moisture content greatly influence the amount of methyl bromide sorbed in a fumigation chamber. Dry peat sorbed 41 per cent of the charge of methyl bromide while wet peat sorbed only 20 per cent. Clay sorbed 25 per cent if dry, and less than 12 per cent when wet, and sand sorbed less than 10 per cent when wet or dry. Since peat and moisture content of nursery soils vary considerably, this sorbtion of methyl bromide may partly explain why dosages lower than 4 lbs. per 100 cu. ft. are occasionally reported to be satisfactory, especially in cases where the organic matter in the soils is low. The soil mixture (1) used in these experiments contains 25 per cent peat, and this may account for the poor control of fungi at the lower dosages.

SUMMARY

Gaseous methyl bromide applied at the rate of 4 lbs. per 100 cu. ft. of space for 24 hours gave excellent control of pathogens in soil in nursery flats. Pure cultures of 10 species of fungi and soil infested with *Rhizoctonia solani* and *Fusarium oxysporum* f. *callistephi* were used in the tests. Only microsclerotia of *Verticillium albo-atrum* frequently survived the treatment. No toxic effects due to fumigation with methyl bromide gas were noted on 17 species of plants adjacent to a fumigated area in a greenhouse chamber, nor was the soil toxic to seeds or transplants if aerated 24 hours before using. The gas killed sclerotia of *Sclerotium delphinii* buried 30 in. in a clay loam soil in 1 test, but only to 18 in. in a sandy loam in another test. Dosages less than 4 lbs. per 100 cu. ft. were found to be unsatisfactory for control of *Rhizoctonia* or *Fusarium* in soil of high organic matter content. The amount of organic matter and moisture content of soils is known to influence the amount of methyl bromide sorbed by soils, and this fact may account for apparent successful fumigation of soil with lower dosages of methyl bromide.

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THE RELATION OF ASCOCHYTA IMPERFECTA TO ALFALFA SEED PRODUCTION IN MINNESOTA[1]

M. F. Kernkamp and G. A. Hemerick

Although Minnesota holds a leading position in the production of alfalfa for forage, at least a million acres being devoted to the crop, the production of seed in the state has declined during the past 20 years to negligible quantities. All the reasons for the decline are not known, but it is a fair assumption that diseases play a role in the reduced production of seed.

Foliage of alfalfa in Minnesota is subject to a variety of diseases. Of these, black stem, caused by *Ascochyta imperfecta* Pk., appears to be one of the most destructive. This paper presents results of studies on that pathogen, especially in relation to seed production, and on methods of determining losses caused by black stem.

LITERATURE REVIEW.—The morphology, physiology, and pathogenicity of *Ascochyta imperfecta* and the symptoms of black stem on alfalfa leaves and stems have been reported by many workers (2, 6, 10, 11, 13, 14). Cormack (2) showed that *Ascochyta imperfecta* is seed-borne, but he suggested that it "persists mainly as mycelium on or in the outer portion of the seed-coat." He found the fungus fruiting on overwintered stems of alfalfa but not on seeds.

The inoculum of the black stem fungus and related fungi may remain viable in the seed and dry stems of their respective hosts, but it usually does not persist more than 2 years in the soil in the absence of the host (2, 4). Seed treatment has consistently failed to control black stem (2) and related diseases because the organisms infect the living embryos (3, 8). Neither can mild seed infection be detected by casual observation, since infected seedlings frequently result from apparently noninfected seeds (2, 3, 7).

The nature of seed loss of alfalfa was studied in Minnesota, Saskatchewan, and Utah by Hughes (5), Bolton and Peck (1), and Sorenson (12), respectively. In these studies the yield of alfalfa seed was low in certain fields because a large proportion of the seeds, being light in weight, were blown out of the thresher along with the straw and chaff. Light seeds were described as shrivelled, dark brown, and dead. Weather and insect damage were suggested (1) as possible causes of the shrivelling. The writers (9) published a preliminary report in which they pointed out that *Ascochyta imperfecta* is one of the factors that reduce alfalfa seed yields.

OBSERVATIONS AND EXPERIMENTAL RESULTS.—During the course of this study at least 500 cultures of *Ascochyta imperfecta* were isolated from various parts of the inflorescence and foliage of alfalfa plants collected in virtually every region in Minnesota where alfalfa is grown. Of these isolates, 20 were selected to represent diverse cultural types and were compared in pathogenicity on 69 different clones of alfalfa. They appeared equally pathogenic on all the clones, although they differed in cultural appearance and produced cultural variants. All the isolates were easily recognizable as typical cultures of *A. imperfecta;* it was the only fungus found parasitizing alfalfa and seeds in this study.

Inoculation tests in the greenhouse also demonstrated that these isolates were pathogenic on 19 other species of legumes: *Arachis hypogaea* L., peanut; *Cajanus indicus* Spreng., pigeon pea; *Clitoria mariana* L., butterfly pea; *Cyamopsis tetragonoloba* Taub., guar; *Medicago falcata* L., yellow alfalfa; *M. lupulina* L., black medic; *M. sativa* L., alfalfa (lucerne); *Melilotus alba* Desr., white sweet clover; *M. indica* (L.) All., sour clover; *M. officinalis* (L.) Lam., yellow sweet clover; *Phaseolus aureus* Roxb., mung bean; *P. calcaratus* Roxb., rice bean; *P. vulgaris* L., navy bean; *Pisum arvense* (L.) Poir., common field pea; *Stizolbium deeringianum* Bort., velvet bean; *Trifolium hybridum* L., alsike clover; *T. incarnatum* L., crimson clover; *T. pratense* L., red clover; *Vicia americana* Muhl., common vetch; *Vigna sinensis* (L.) Endl., cowpea. These results suggest that other hosts may be important in the epidemiology of black stem on alfalfa.

The inoculum used in field and greenhouse tests usually was a composite of at least 100 recent isolates from different geographic areas and different plant organs. Inoculum was produced on potato-dextrose agar in Petri plates. Sporulation declined rapidly at temperatures above 23° C.; it was therefore necessary to incubate cultures at that temperature during the summer when the inoculum was needed for field experiments. Sporulation was also best when the agar was buffered to pH 5.7. This was accomplished by the addition of approximately 5 gms. of dibasic potassium phosphate to a liter of melted agar prior to sterilization. Spores were washed off the agar and applied to plants by means of an atomizer, knapsack sprayer, or power sprayer.

The symptoms of black stem on alfalfa that were observed in these studies agree with those reported in the literature (2, 3, 6, 10, 13, 14). In the early stages of infection, small black lesions appeared on the leaves and stems. As they progressed, those on the leaves caused profuse defoliation. Stem lesions enlarged and coalesced during the season until the entire stem became surrounded by a black sheath of fungus mycelium and diseased host tissue. At this stage all the leaves usually had dropped, peduncles and floral parts were infected, and flowers frequently had fallen. Inoculations in the greenhouse conclusively demonstrated the symptoms on fruiting structures and the

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effects of seed production.

GREENHOUSE TESTS.—Plants were inoculated in 3 replicate tests at intervals of 30 days. In each test the flowers on 18 alfalfa plants were pollinated by hand daily for 14 days. Twelve of the plants were inoculated by atomizing with a spore suspension of *Ascochyta imperfecta*, and 6 control plants were atomized with water only. The plants were incubated under plastic sheeting, out of direct sunlight, for 3 days at 21–24° C., then returned to the greenhouse bench.

Severe symptoms of black stem developed on the stems and leaves of the inoculated plants in 7 days, and the flowers, buds, peduncles, and pods became infected. Dark lesions developed on the receptacles and pedicles of young pods within 5 days; older pods became necrotic in 10 days. Thirty days after pollination and inoculation the infected pods were flat, dry, and straw colored, resembling mature pods.

The noninoculated controls remained free from disease and developed normal, heavy, plump, yellow, and viable seed (Figure 1). All the seed was harvested and saved for further analysis.

Seeds from infected plants were uniformly light in weight, brown, shrivelled, and nonviable (Figure 1), similar to seed produced in the field on plants infected with black stem. While the pods were dying and drying out, their stems also became necrotic, dry, and bleached.

These experiments proved beyond any doubt that *Ascochyta imperfecta* can virtually destroy a seed crop of alfalfa if sufficiently heavy infection occurs at the flowering stage.

FIELD EXPERIMENTS.—Since greenhouse tests demonstrated that black stem could virtually eliminate production of alfalfa seed by inoculated plants, attempts were made to duplicate the experiments in the field. These attempts failed because there was always so much natural infection in the checks that no comparison could be made.

A second approach was to control black stem in certain plots by spraying or dusting them with fungicides, and then to compare yields from sprayed and dusted plots with those from plots in which the disease was allowed to develop naturally. These experiments also failed because it was impossible to control the disease with any of the fungicides used.

The experiments with fungicides were extensive enough to deserve description, even if results were negative. During the summer of 1950, replicated plots, each nearly an acre in size, in commercial fields of alfalfa in Lake of the Wood County, Minnesota, were dusted with ziram (76 per cent zinc dimethyldithiocarbamate), zineb (65 per cent zinc ethylene bisdithiocarbamate), nabam 65 per cent disodium ethylene bisdithiocarbamate), ferbam (70 per cent ferric dimethyl dithiocarbamate), and tri-basic sulfate. Applications were made every third day with a commercial-type potato duster at rates of 30 lb. of dust per acre. At the Agricultural Experiment Station, Rosemount, Minnesota, the same fungicides were applied as sprays each week. None of the fungicide applications reduced the severity of black stem.

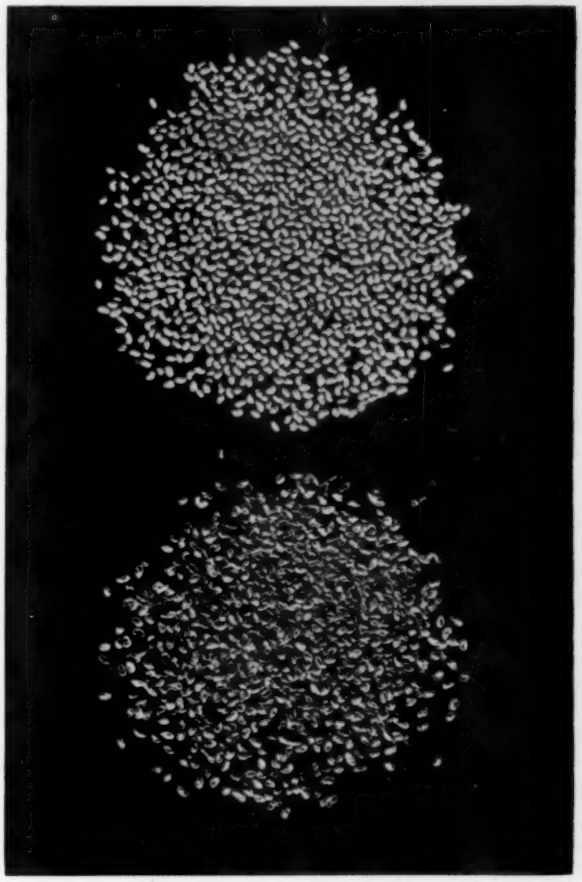

FIG. 1. Alfalfa seeds: top, from plants infected with *Ascochyta imperfecta;* bottom, from noninfected plants.

Since these fungicides were ineffective, 88 other common fungicides were tested for their toxicity to *Ascochyta imperfecta* as well as to alfalfa. The following 6 fungicides alone showed promise: Arasan, (50 per cent tetramethyl thiuram disulfide), Phygon (50 per cent 2,3-dichloro-1,4-naphthoquinone), Spergon (96 per cent tetrachloroparabenzoquinone), Semesan (30 per cent hydroxymercurichlorophenol), Tersan (50 per cent tetramethyl thiuram disulfide), and Ceresan (2 per cent ethyl mercury chloride) were selected. Their effectiveness was tested on field plots during the summer of 1951. Again black stem was not controlled, and seed yields from plots sprayed with these fungicides and nonsprayed plots were equal.

An attempt was made to control black stem by destroying overwintered inoculum by cultivation in field plots 20 ft. square, and comparing yields from these plots with yields from adjacent plots with abundant inoculum. Black stem was severe in all plots, so these experiments failed.

SEED ANALYSIS.—Since these attempts were unsuccessful, it became necessary to devise other methods of analyzing seed loss caused by *Ascochyta imperfecta*.

TABLE 1.—*Quantitative characteristics of seeds in light, intermediate and heavy classes*

Characteristics	Seed-weight classes					
	Light		Intermediate		Heavy	
	Range	Mean	Range	Mean	Range	Mean
Seed weight (mg.)	0.25–1.49	0.75	1.50–1.99	1.62	2.00–7.50	2.22
No. seeds per gm.	4000–670	1333	669–500	613	499–133	450
No. seeds per cc.	1100–480	693	479–390	464	389–120	370
Density of samples:						
Grams per cc.	0.27–0.71	0.52	0.72–0.77	0.75	0.78–0.92	0.82
Pounds per bu.	21–55	40	56–59	58	60–72	64

Attempts were made, therefore, to analyze seed from the greenhouse tests and from fields having severe and light infection of black stem. Analyses were based on weight of seeds, density of samples, percentage of nonviable seed, percentage of seeds infested with *A. imperfecta*, and percentage of emergence of seedlings from seeds planted in soil.

As stated previously, the seed was harvested from the severely infected and noninfected plants in the greenhouse. Samples of mature seed pods were collected from 24 fields in which black stem was either severe or very light, in northern Minnesota, from University Farm, St. Paul (light infection), and from Rosemount (severe infection).

When dry, the pods were threshed and cleaned by hand to insure saving all the seeds formed, regardless of quality. A composite sample to represent all 24 fields in northern Minnesota was made, and composite samples of seed were prepared from infected and noninfected plants, respectively, in the greenhouse.

Seeds in each of the 3 composite samples were separated into seed-weight groups by means of an air blast seed separator. The groups were separated at intervals of 0.25 mg. per individual seed, the lightest being 0.25 and the heaviest 7.50 mg. per seed.

Seeds from plants infected with *Ascochyta imperfecta* weighed less than 1.5 mg. per seed; those from noninfected plants weighed more than 2 mg.; almost no seed produced in the greenhouse weighed between 1.5 and 2 mg.

In the composite of seeds from fields most weighed either less than 1.5 mg. or more than 2 mg., but some weighed between 1.5 and 2 mg. The field seeds in each of the 3 classes were analagous to their counterparts from the greenhouse, and the seed in each class appeared homogeneous. Thus 3 classes were established: light, intermediate, and heavy, with mean weights of less than 1.5 mg., between 1.5 and 2 mg., and more than 2 mg. All the samples from individual fields were then separated and classified accordingly.

The quantitative characteristics of seeds in each of the 3 classes are in Table 1. To acquire these data, 10,000 seeds from each weight class were counted, and calculations were based on mean weight of individual seeds from each class. Number of seeds per gram and number of seeds per cc. were determined; grams per cc. and pounds per bushel were calculated.

MEASUREMENT OF SEED LOSS.—*Seed weight as a criterion of seed loss.*—Seeds which weigh less than 1.5 mg. are usually blown out of the threshing machine with the straw and chaff; they do not usually remain in samples of commercial seed. Therefore, of the total number of seeds produced, the percentage which weighs less than 1.5 mg. is a measure of crop loss. In 1 severely infected field at the Agricultural Experiment Station, Rosemount, Minnesota, 80 per cent of the seeds were "light"; thus one can assume an 80 per cent reduction of seed in that case.

Bushel weight as a criterion of seed loss.—Bushel weight often expresses the quality of certain crops. On the basis of data in Table 1, bushel weights were determined for seed from the various sources in these studies. The bushel weights of seed from infected and noninfected plants in the greenhouse and from fields with high and low severity of black stem are in Table 2.

TABLE 2.—*Bushel weight of alfalfa seed from plants with different degrees of black stem infection*

Source of Seed	Infection	Pounds per bushel
Greenhouse (artificial inoculation)	Severe	36
Rosemount, Minnesota	Severe	35
Northern Minnesota	Moderate to heavy	45
Northern Minnesota	Light	60
Greenhouse (noninoculated)	None	66

A bushel of seed from infected plants in the greenhouse weighed 36 lb., and a bushel of seed from severely infected fields weighed 35 lb. A composited sample of seed from 12 fields having moderate to severe infection weighed 45 lb. per bushel, while seed from fields with light infection weighed 60 lb. per bushel; seed from noninoculated plants in the greenhouse weighed 66 lb. per bushel.

These data indicate clearly the depreciation of the value of seed from infected fields as compared with noninfected fields. The standard weight of alfalfa seed is 60 lb. per bushel; 45 lb. seed is very poor quality, and 35 lb. seed is virtually worthless.

Number of seeds infected with Ascochyta imperfecta.—Another method of determining the relationship of seed quality to infection by *Ascochyta imperfecta* is to

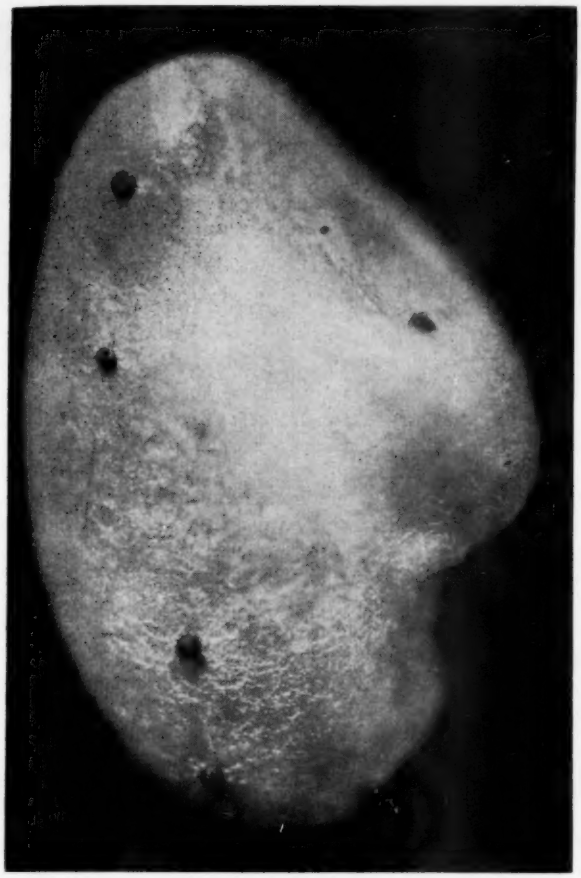

FIG. 2. Pycnidia of *Ascochyta imperfecta* on an alfalfa seed.

determine the number of seeds actually infected with the organism. This was done with all the samples of seed from the greenhouse and from fields having light to severe infection. Also the numbers of infected seeds were determined for the light, intermediate, and heavy seed classes regardless of source.

The percentage of infected seeds was determined in 2 ways: 1) by surface sterilizing the seeds, plating them on potato-dextrose agar, and counting the number of colonies of *Ascochyta imperfecta* that grew from them, and 2) by placing nonsterilized seed on wet blotters in Petri dishes and counting the number of seeds that bore pycnidia (Figure 2) after 7 days at 21–24° C.

Percentages of infected seeds were based on 4 replicates of 100 seeds from each sample. Along with these tests, the percentage of seeds infected with Ascochyta were determined for several commercial samples obtained from the Minnesota State Seed Laboratory.

The percentages of infected seeds for each weight class in relation to degree of foliar infection are in Figure 3. It is obvious that the number of light seeds is directly proportional to the degree of foliar and seed infection, but the number of infected seeds was inversely proportional to the weight of the seeds. Nearly 100 per cent of the light seeds from Rosemount were infected, whereas only 46 per cent of the heavy seeds from Rosemount were infected. Only 60 per cent of the seeds from the severely infected plants in the greenhouse were infected, because the peduncles became infected, resulting in light, chaffy seeds that were not infected directly.

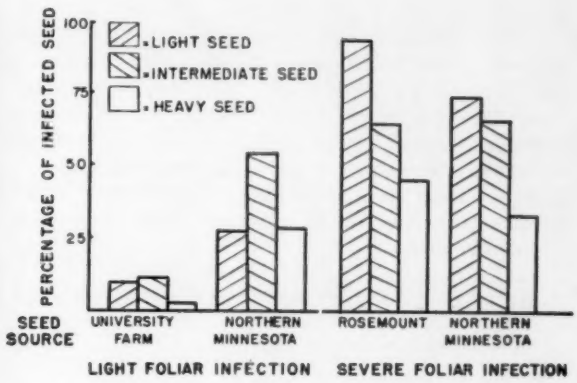

FIG. 3. The percentage of seeds infected with *Ascochyta imperfecta* for each weight class in relation to the degree of foliar infection.

The seed from California was free of *Ascochyta imperfecta*. Five, 8, and 13 per cent of the seeds from Elbow Lake, Alexandria, and Williams, Minnesota, respectively, were infected with *A. imperfecta*. Forty-nine and 77 per cent of the seeds from Dawson and Hoffman, Minnesota, respectively, were infected. The latter came from areas where black stem was epidemic.

Percentage germination of seed.—Since these results and others (2, 8) had shown that *Ascochyta imperfecta* is seed-borne, it became apparent that this might have some influence on subsequent germination of that seed. The viability of seed from sources having different degrees of infection was determined as a possible criterion of the association of the organism with seed quality.

Percentage germination was measured by means of 400 seeds each selected from light, intermediate, and heavy classes from samples of different origin. The seeds were germinated on wet blotters in Petri dishes at 21–24° C., and seedlings were counted after 7 days.

Percentage germination of seeds by weight class and source is in Table 3. Few light seeds were viable, and germination of the intermediate seeds was intermediate between the light and heavy seeds.

A preliminary experiment was made on the emergence of seedlings from seed samples that had known

TABLE 3.—*Percentage germination of light, intermediate, and heavy seed from various sources*

Light Seeds	Intermediate Seeds			Heavy Seeds	
All sources	U. Farm	Northern Minn.	Green-house	Northern Minn.	U. Farm
2	43	65	79	81	87

percentages of seed infection with *Ascochyta imperfecta*. Four hundred seeds from each sample, replicated 4 times, (100 seeds per replicate) were planted in nonsterilized soil, and stands were counted 15 days later. The stands are given in Table 4. Stands from commercial seeds with a high degree of infestation of *A. imperfecta* were very low compared with seed samples having a low incidence of infestation. This is further indication that *A. imperfecta* reduced the yields of salable seed.

TABLE 4.—*Emergence of seedlings from 5 lots of seed with different degrees of infestation of Ascochyta imperfecta and planted in nonsterilized soil*

	Source of Seed				
	Western Minn.	Rosemount Minn.	Northern Minn.	Western Minn.	California
Percentage of infestation	78	65	13	5	0
Percentage of stand	12	17	40	35	58

SUMMARY AND CONCLUSIONS

Greenhouse tests demonstrated conclusively that *Ascochyta imperfecta* can virtually eliminate production of seed if alfalfa plants are inoculated during the fruiting stage. Although it was not possible to compare directly the yields from inoculated and check fields plots, the evidence obtained from comparing severely infected and slightly infected fields showed that black stem also reduced yields in the field. Other agents may be involved in seed losses in the field, but none was apparent in these studies.

Since direct comparisons of yields from inoculated and noninoculated fields plots could not be made, seed was harvested by hand from fields with little or no infection and from fields with severe infection of black stem. With seed from greenhouse tests as a basis of comparison, seeds from selected fields were analyzed for weight of individual seeds, weight per bushel, percentage of seeds infected with *Ascochyta imperfecta*, percentage of seeds that germinated in blotter tests, and percentage of seeds that produced seedlings when planted in soil. On the basis of weight, seeds fell into 3 classes, light, intermediate, and heavy, with mean weights of 0.75, 1.62, and 2.22 mg. per seed, respectively. All the seed harvested from inoculated plants in the greenhouse were light. All the seed harvested from control plants in the greenhouse were heavy. Seed harvested from field plots fell into all 3 catagories, depending on the degree of infection with *A. imperfecta*.

When seeds from fields were analyzed, direct correlations were found between numbers of light seeds and severe infection in fields, numbers of light seeds and seeds infected with *Ascochyta imperfecta*, and numbers of seeds infected with *A. imperfecta* and germination and stands.

Light chaffy seeds that weigh less than 1.5 mg. are blown out of a threshing machine and are found only in small numbers in commercial seed. Also seeds in the intermediate and heavy classes are frequently infected with *Ascochyta imperfecta*, but it is not apparent until the seeds are germinated on wet blotters and the fungus is allowed to fruit (Figure 2).

These results not only demonstrate that actual yields are reduced by black stem, but the quality is greatly impaired through reducing viability and carrying the organism. It would appear that *Ascochyta imperfecta* as carried in the seed is extremely important in initiating seedling blight and seasonal epidemics caused by the same organism.

Black stem apparently cannot be controlled at this time by the use of chemical sprays and dusts, but several cultural practices may be suggested. Crop rotation will reduce disease because it takes at least 2 seasons to build up enough inoculum for a severe epidemic. Burning and cultivating fields early in the spring to eliminate overwintering inoculum tend to delay the development of epidemics. The latter 2 methods, however, are not recommended because burning is dangerous and destroys much organic matter. Cultivation wounds the crowns and roots of plants and increases the incidence of crown and root rot, thus reducing stands.

The use of resistant varieties appears to be the only practical means of control, but at the present time there are no resistant varieties. Recently, however, the writers have found indications that there are resistant clones of alfalfa. If this proves to be true, those clones will be incorporated into a program of breeding for resistance to *Ascochyta imperfecta*.

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THE VAPOR PHASE TOXICITY OF SOME BIPHENYL DERIVATIVES TO MONILINIA FRUCTICOLA [1]

Louis Beraha and Dwight Powell [2]

A fungicide which exerts its action in the vapor phase is by no means novel. The vapors of benzol and paradichlorobenzene effect control of the tobacco downy-mildew fungus, *Peronospora tabacina*, by solution in the water either on or within the host-plant tissues, and in constituents of living cells of the tobacco plant and the mycelium of the fungus (15). Similarly, the commercial seed-treatment preparations, Ceresan and New Improved Ceresan, emit fungicidally-active vapor (7). Biphenyl vapors emitted from impregnated wrappers inhibit the growth of certain citrus fruit pathogens (11). The action of soil fumigants has mostly been limited to destruction of nematodes and wireworms although they may act on certain fruiting structures of the soil fungi (6). Reports of chemicals that exert their fungicidal action in the vapor phase have for the most part been limited to *in vitro* laboratory studies. Very few of these chemicals have been used as practical disease control measures (5), (13), (10), (9), (14), (12), (16).

Preliminary field studies in 1950 on Elberta peaches indicated that certain biphenyl derivatives were toxic at very low concentrations to *Monilinia fructicola* (Wint.) Honey but the treatments resulted in off-flavored fruit. The purposes of this study were to formulate some essential concepts of the mode of action of these toxicants, and to correlate the molecular configuration of promising biphenyl compounds with their toxicity to the test organism.

METHODS AND MATERIALS.—Spores of *Monilinia fructicola* were obtained from 7-day-old cultures grown on potato-dextrose agar. Spore suspensions were made with a sucrose-citric acid solution (8), and were regulated in each case to approximately 50,000 spores per ml. Winesap and Golden Delicious apples were used for many of the tests since previous studies had indicated that their reactions were both consistent and well correlated with that of the peach.

Tissue plugs were removed from apples with a sterilized 1 cm. cork borer and 6 individual plugs of equal size were distributed on glass cover slips in chemically-clean Petri dishes containing moistened filter paper. The test chemical was placed on a glass cover slip in the center of the Petri dish. Inoculations of the apple plugs were made by pipetting .05 ml. of a spore suspension on the surface 24 hours prior to exposure to the chemical. Petri dishes were sealed with cellulose tape and after 48–72 hours, records were made as to degree of rotting and extent of surface sporulation.

Secondly, 2 Van Tiegham cells were mounted on a single glass slide with paraffin and the chemical placed within the cells. .05 ml. of spore suspension was pipetted on a second slide in areas bordered by a circular, fine line drawn with a wax pencil. This slide was then inverted and sealed by petroleum jelly over the Van Tiegham cells with the sublimating chemical. The percentage of spore germination was recorded after 16–18 hours.

In a third test, the calyx area of the apple was inoculated by injuring the skin with a sharp needle and immersing the injured area into a spore suspension wetted with Santomerse S. In other apples, plugs were removed and inoculations were made at the base of the plug. The plugs were then replaced and sealed in the region of the incision, with petroleum jelly. These apples were then transferred to a 21 × 7½ cm. humidity chamber containing moistened hand towels. The toxicants were dissolved in an organic solvent and absorbed on Whatman No. 1 filter paper. After the solvent had evaporated, the impregnated filter paper was placed in the moist chamber in close proximity to the inoculated apples. Temperatures were maintained at 75° ± 3° F. Records of rotting of the fruit and sporulation of the fungus were made 7 days later.

RESULTS.—In the tissue plug test conducted in sealed Petri plates (Table 1) o-fluorobiphenyl, o-chlorobiphenyl, p-chlorobiphenyl, biphenyl, and polyethyl biphenyl prevented rotting at 250 p.p.m. Both o-fluorobiphenyl and biphenyl were effective at 160 p.p.m. whereas p-chlorobiphenyl, o-chlorobiphenyl, and polyethyl biphenyl manifested slight toxicity. The following compounds were not effective: o-aminobiphenyl, N,N′-di(o-biphenyl) thiourea, o-nitrobiphenyl, p-ami-

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Appreciation is extended to the Monsanto Chemical Company, St. Louis, Missouri, for supplying the compounds used in this study.
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TABLE 1.—*Evaluation of some volatile chemicals against M. fructicola on apple tissue plugs after 72 hours exposure*

Chemical	Concentration in p.p.m. per volume of air in sealed Petri dish [a] 160	250
o-fluorobiphenyl	xxx	xxx
o-chlorobiphenyl	xx	xxx
p-chlorobiphenyl	xx	xxx
biphenyl	xxx	xxx
2-methoxybiphenyl	x	xx
polyethyl biphenyl 2100	xx	xxx
polyethyl biphenyl 2400	xx	xxx

[a] x—rotting and sporulation; xx—rotting and no sporulation; xxx—slight rot no sporulation.

nobiphenyl, 2-nitro-4′-chlorobiphenyl, o-terphenyl, N-(2-biphenyl)-stearamide, 4,4′-dinitrobiphenyl disulfide, biphenylacetonitrile, p-cinnamoyl-biphenyl sulfide, biphenyl disulfide, 4-4′-biphenyldisulfonyl difluoride, 2-ethylhexyl biphenyl phosphate, x,x-diisopropyl biphenyl polyisopropyl biphenyl polyethylpolyisopropyl biphenyl, polyethylpolyisopropyl biphenyl 4324, polyamylbiphenyl, polyamylbiphenyl 3500, amylchlorobiphenyl 1010, x-amyl-4,4′-dichlorobiphenyl, x,x′-dihydroxyoctachlorobiphenyl, x,x′-dihydroxyoctachlorobiphenyl, dicuprous salt, and 3,3′-bis(trifluoromethyl) biphenyl.

To determine the effects of short exposures, inoculated apple plugs were exposed to biphenyl 500 p.p.m. for periods ranging from 1 to 5 hours. At hourly intervals the chemical toxicant was removed and the plugs were observed for an additional period sufficient to cause rotting and sporulation in the control. After periods of 48 and 72 hours, plugs from the 1- and 2-hour exposures showed normal infection. Plugs from the 3-, 4-, and 5-hour exposures were delayed 24–30 hours in showing symptoms of rot. The delay in rotting and sporulation was proportional to the length of exposure. Thus, biphenyl acted fungistatically on short exposures and spores readily germinated when associated with a nutritive base. Long exposures, however, prevented rotting and sporulation on the surface which indicated that a lethal concentration of the toxicant had permeated the tissues.

TABLE 2.—*Percentage inhibition of M. fructicola spores in Van Tiegham cell in relation to chemical exposure*

Chemical	Concentration in p.p.m. per volume of air in Van Tiegham cell 2,500 p.p.m.	50,000 p.p.m.
o-fluorobiphenyl	100	100
p-aminobiphenyl	22	83
o-chlorobiphenyl	92	98
p-chlorobiphenyl	18	50
p-nitrobiphenyl	10	27
2-amino-4′-chlorobiphenyl	0	8
2-nitro-4-chlorobiphenyl	80	94
biphenyl	97	97
2-methoxybiphenyl	10	32
polyethyl biphenyl 2100	—	75
polyethyl biphenyl 2400	—	80
control	0	—

In Table 2 are listed the results when spores in suspension were exposed in Van Tiegham cells to the more effective compounds at 2500 and 50,000 p.p.m. for periods of 16–18 hours. o-fluorobiphenyl was the only chemical to give complete spore inhibition at a concentration of 2500 p.p.m. Biphenyl was second in toxicity at both concentrations. o-chlorobiphenyl was third with 92 per cent inhibition and was more effective than p-chlorobiphenyl. 2-nitro-4-chlorobiphenyl inhibited 80 per cent of the spores but did not show this toxicity in the apple plug test. Even at 50,000 p.p.m., o-fluorobiphenyl was the only material to give complete inhibition.

Again in determining the effect of short exposures of the sublimating chemicals, spores were exposed to biphenyl (2500 p.p.m.) in Van Tiegham cells for periods ranging from 1 to 4 hours. After a 1-hour exposure plus 3 hours to complete the germination process, germination was normal with normal germ tube length as compared to the check. After a 2-hour exposure plus 2 hours unexposed, germination was practically normal but germ tube length was shorter and cell growth was definitely affected. After a 3-hour exposure plus 1 hour unexposed, germination was definitely inhibited but not entirely curbed. After a 4-hour exposure there was no germination.

All these samples were again exposed an additional 15 hours. Ten per cent of the spores with the 4-hour exposure finally germinated.

When injured apples were inoculated and placed in a humidity chamber with impregnated filter paper containing the toxicants, o-fluorobiphenyl, o-chlorobiphenyl, and biphenyl were the most effective in preventing fruit rot (Table 3). p-chlorobiphenyl limited rotting and prevented sporulation at 4 and 16 p.p.m. 2-methoxy biphenyl reacted similarly at 16 p.p.m.

Apples uniformly inoculated at the base of a plug with several drops of a spore suspension or with mycelia inoculum and exposed to biphenyl (2 p.p.m.) for 72 hours manifested slight rotting in the vicinity of the plug. Cuts through these apples revealed a limited rotted zone radiating from the original site of the inoculum. No sporulation was apparent on the surface of these fruits. The limited rotting and lack of sporulation would indicate that the toxicant was absorbed by the apple. That some rotting did occur may have resulted from the time lag before the toxicant reached the infected area. These apples were definitely off-flavor. Transfers of the apples to toxicant-free chambers did not alter their off-flavor nor allow the resumption of fungal activity.

Four-day-old colonies of *M. fructicola* grown on P.D.A. in Petri plates were exposed to the sublimating toxicants by placing 25, 50, and 100 mg. of the test chemical on sterile cover slips 5 cm. from the edge of the colony. Measurements made at daily intervals in comparison with the controls showed that the rate

TABLE 3.—*Degree of rotting and sporulation on surface of apples exposed to volatile fungicides* [a]

Chemical	Concentration in p.p.m. per volume of humidity chamber		
	2 p.p.m.	4 p.p.m.	16 p.p.m.
	Type of growth [b]		
o-fluorobiphenyl	xxx	xxx	xxx
o-chlorobiphenyl	xxx	xxx	xxx
p-chlorobiphenyl	x	xx	xx
biphenyl	xxx	xxx	xxx
2-methoxybiphenyl	x	x	xx
polyethyl biphenyl 2100	x	x	x
polyethyl biphenyl 2400	x	x	x
control	x	x	x

[a] The rotting of the fruit was determined as due to *M. fructicola* by tissue plating from edge of rotted area.

[b] x—rotting severe, sporulation on surface; xx—rotting limited, no sporulation on surface; xxx—no rotting, no sporulation on surface.

of spread was markedly curtailed. In some cases, the borders of the fungal colony were blackened and appressed. Sporulation and mycelial development were abnormal. Results indicated that o-fluorobiphenyl, o-chlorobiphenyl, p-chlorobiphenyl, and biphenyl were fungistatic since replating allowed continuance of growth whether portions had been removed from either the center or edge of the colony.

In the 3 different procedures used it was necessary to vary drastically the concentration of the chemicals. The low concentrations in the apple inoculation test were ineffective in the plug test. Presumably in the latter method a more suitable medium for fungus growth was provided. In evaluating these results only the degree of rotting was observed. Where it was possible actually to observe germination of the spores as in the Van Tiegham cell, effective concentrations ranged from 2500 to 50,000 p.p.m. Thus, while germination of the spores undoubtedly occurred in the apple tissue inoculations, rotting could be inhibited at such low concentrations as 2 p.p.m. (Table 3).

DISCUSSION.—Biphenyl derivatives of low molecular weight were extremely effective against the brown rot organism *in vitro* and *in vivo* and in subsequently preventing rot of susceptible tissue. This toxicity is apparently related to the high vapor-pressure inherent in such compounds. Single halogen derivatives were more effective than those which possessed nitro, amino, or alkyl substituents. The effectiveness of o-chlorobiphenyl, and o-fluorobiphenyl as compared to p-chlorobiphenyl suggests that halogen substitution in the ortho position results in greater fungitoxicity than those in the para position. The action of these materials at continual exposure demonstrated their effectiveness at very low concentrations. The order of sensitivity of the fungus to these biphenyl derivatives was nearly the same in each case utilizing *in vitro* or *in vivo* methods of testing their effectiveness. While the effects on nuclear structure of the mycelium and spores were not investigated, stunting with the abnormal production of crosswalls in the conidial germ tubes was noted. These abberations may in fact indicate a cytological effect similar to that caused by the benzene derivatives (3) (4).

Exposures equivalent to ¼ time of the germinating process of a standard suspension of *M. fructicola* spores were ineffective. Some effects were shown, however, when the exposure time was for at least ½ of the germinating period. This may be explained on the basis of time necessary for diffusion of the vapor through the cell wall of the spores, transfer of the vapor to the site of action, and finally concentration of the vapor in amount sufficient to produce a lethal effect.

The effect of arresting mycelial development after a second exposure demonstrated that these toxicants act on the same or nearly same physiologic or nuclear systems required for the germination of spores and elongation and maturation of mycelium. Physiological differences between the spore and mycelium in the case of *M. fructicola* are not indicated as is claimed for *Botrytis* by Rich (12).

SUMMARY

In the Van Tiegham cell, o-fluorobiphenyl at 2500 p.p.m. completely inhibited spore germination of *Monilinia fructicola*. Apparently both percentage of germination and length of the germ tubes were inversely proportional to the time of exposure. In apple tissue plugs, o-fluorobiphenyl, o-chlorobiphenyl, p-chlorobiphenyl, biphenyl, and polyethyl biphenyl were toxic in the vapor phase at 160 p.p.m. as evidenced by the lack of rot after 72 hours.

When inoculated apples were exposed to the vapor toxicants, at 2 p.p.m. for 72 hours, o-fluorobiphenyl, o-chlorobiphenyl, and biphenyl were the most effective in preventing rotting of the fruit. p-chlorobiphenyl allowed limited rotting but prevented sporulation. However, the effective compounds were shown to be fungistatic since replatings from exposed tissues allowed continuance of growth.

Halogen derivatives were more toxic than those which contained the nitro, amino, or alkyl substituents, but all the effective compounds caused an off-flavor of the fruit.

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A PHYTOPATHOLOGICAL NOTE

Hormodendrum Kernel Rot in Dent Corn. Paul E. Hoppe.[1] An unusually heavy incidence of rot following injury to the kernels was observed in a field of hybrid corn in southwestern Wisconsin in 1952. Approximately 5 per cent of the ears and from less than 1 per cent up to 25 per cent of the kernels in different ears were affected. Another hybrid growing in an adjacent field showed no evidence of this disease.

Platings of diseased kernels from many ears yielded, in every instance, a fungus with cultural characteristics resembling *Penicillium*, but which upon microscopic examination was identified as a species of *Hormodendrum* Bon. The more common wound parasites, such as *Fusarium moniliforme* Sheld., *Penicillium* spp., etc., were conspicuously absent in these platings.

The kernel wounds consisted of breaks in the pericarp, usually in the center of the crown and at right angles to the longer dimension. Damage ranged from a superficial darkening along margins of the wounds to nearly complete rotting of the kernel (Fig. 1). Where infection was severe the entire crown was black as though charred by fire, giving the ear rather spectacular appearance. Dingy discolored husks suggested the probable path of fungus penetration.

The actual feeding value of the crop was only slightly reduced by this unusual disease. The "eye appeal" factor, however, is important in corn hybrids. Blemishes that detract from a showy appearance are discriminated against by farmers and seedsmen alike. This injury appeared to be an autogenous break influenced by the genetic makeup of the hybrid and expressed under certain conditions of environment. Unfortunately, these conditions may not occur until after a susceptible hybrid has been released for commercial production.

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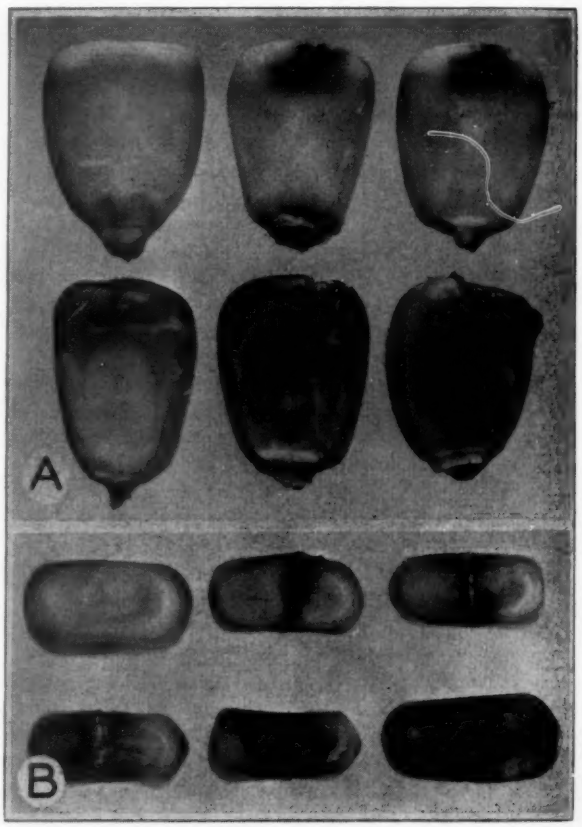

Fig. 1. Varying degrees of *Hormodendrum* infection following kernel injury in dent corn. A) flat surface views of healthy and diseased kernels; B) crown views.

The writer knows of no previous reports of *Hormodendrum* occurring so extensively as a wound parasite in corn and to the exclusion of the fungi more commonly associated with this type of kernel disease.—Wisconsin Agricultural Experiment Station, University of Wisconsin, Madison, Wisconsin.

PROPERTIES OF THE WOUND-TUMOR VIRUS [1]

Myron K. Brakke,[2] Karl Maramorosch [3] and L. M. Black [2]

The wound-tumor virus, *Aureogenus magnivena* Black (11), induces vein enlargement as the most constant symptom on a wide variety of plants. On many of these it incites root tumors; on a few it induces stem tumors (3, 4). The neoplasms show properties typical of most tumors; wounds seem to play a part in their initiation (5), the heredity of the host is important (7), and the hyperplastic tissue will grow indefinitely if kept on a suitable substrate (6, 10), or if grafted from plant to plant as scion tumors in series (6).

The virus necessary for the formation of these tumors belongs to the group of leafhopper-transmitted plant viruses which have long incubation periods in their insect vectors. Only 1 virus of this group, the potato yellow-dwarf virus (*Aureogenus vastans* (Holmes) Black), can be easily transmitted mechanically from plant to plant. Consequently very little is known about the physical and chemical properties of these viruses.

However, as with some other members of this group (2, 14, 15), the wound-tumor virus can be transmitted from cell-free extracts to a leafhopper vector by injection with a fine glass needle (13). This technique has been used in the determination of some of the properties of the wound-tumor virus and in the demonstration of its filterability through bacteria-retaining filters. A preliminary report of some of the results has been published (9).

MATERIALS AND METHODS.—*Sources of virus.*—The viruliferous leafhoppers *Agallia constricta* Van Duzee or *Agalliopsis novella* (Say) used in the experiments reported here had acquired the virus by feeding on diseased crimson clover (*Trifolium incarnatum* L.) for at least a week. A month or more after the start of the acquisition feeding the insects were collected, weighed, cooled and then macerated in a glass mortar and pestle with a small amount of solvent. This and all other laboratory work was done in a cold room kept between −1° and +2°C. Additional solvent was added to give the desired dilution. After clarification by centrifuging at 3400 r.p.m. in an international 1-SB centrifuge for 20 minutes, the supernatant was removed from between the precipitate and surface fat layer and used for experiments.

Diseased plant tissue (usually from crimson clover) was harvested, weighed, washed, cooled for 1–20 hours in the cold room, and then macerated in a mortar and pestle with a small amount of solvent. After the addition of extra solvent to give the desired dilution, the extract was squeezed through cheesecloth and clarified by centrifuging at 3400 r.p.m. for 20 minutes.

Solvents.—In a few early experiments 0.25 M NaCl was used. However, it was discovered that the pH of extracts from insects in this solvent varied considerably. Thereafter, except for some pH experiments, the solvent used was our "solvent 3" which contains 0.225 M NaCl, 0.0067 M K_2HPO_4, 0.0033 M KH_2PO_4 and has a pH of 6.9.

Assay.—The actual injection has been described in detail by Black and Brakke (8). *A. constricta* were injected in all experiments, in most cases 30 insects being injected with each solution whose infectivity was to be determined. Thirty insects from the same cages were kept for controls, being treated in all respects exactly as the test insects except that they were not injected, or, in a few experiments, were injected with solvent 3.

The actual injection of the insects can be easily and rapidly performed. The number of experiments that can be done is limited not by the injection but rather by the work and space required for raising the insects and testing them for infectivity after injection. In the simplest procedure used for testing injected insects and the one used in the last several experiments, each group of 30 insects was placed on a caged alfalfa plant (*Medicago sativa* L. var. Grimm) in a 4-in. pot of soil in which all weed seeds had been killed by steaming. Alfalfa was selected as the nurse plant for the insects during the incubation period because it is immune from the virus. After 3 weeks the survivors were divided into 6 colonies and each colony placed on a caged crimson clover plant with 3–4 leaves in a 2½-in. pot. After an additional 3 weeks the plants were placed in a small room, the cages removed and the plants immediately fumigated with tetraethylpyrophosphate to kill the leafhoppers. Subsequently the plants were kept in the greenhouse for 6 weeks for observation of the development of the disease. This greenhouse was fumigated at weekly intervals to kill any nymphs that might hatch before they could become infective.

It was necessary to control the growing conditions carefully in order to obtain results. The greenhouse was kept as close to 25°–30° C. as possible by manual manipulation, this being the optimum temperature for development of the virus in both insect and plant (12). Artificial lights were used to provide extra illumination on cloudy days and a 15–16 hour day throughout the year. The extra light was particularly important in maintaining vigorous insect colonies.

Single unexplained contaminations in the test plants for the uninjected controls occurred in 2 widely separated experiments, 1 in August 1949 and the other

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TABLE 1.—*Dilution end-point of virus-containing extracts*

Total Number of Experiments	Dilution [a]								Uninjected controls
	10^{0}	10^{-1}	$10^{-1.5}$	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
				Crimson Clover Extracts					
2[b]	4/4	6/10	.....	5/10	1/10	2/10	0/6	.....	0/10
1[c]	5/10	1/10	.....	2/10	0/10	0/10	.....	.....	0/10
				Leafhopper Extracts					
2[b]	.....	.....	.....	.....	5/21	4/21	1/21	0/21	0/21
7[c]	.....	.....	19/68	60/135	34/179	8/170	1/61	0/21	0/181

[a] A dilution of 1:10 refers to an extract whose weight is 10 times that of the source tissue. All dilutions were made with solvent 3.

[b] Insects tested in colonies of 5 or less. Denominator is number of colonies tested, numerator is number of colonies infective.

[c] Insects tested singly. Number tested in denominator, number infective in numerator. Leafhopper data from Black and Brakke (8).

in December 1950. In the summer of 1951 several such contaminations were found; the explanation for this last group of contaminations is known (8). Data from all experiments with contaminated controls were discarded.

The injection of just 1 dilution of each test sample provides only a qualitative assay. This must be borne in mind in interpreting the results obtained. Presence of the virus after a given treatment means that that treatment did not destroy all the virus. It does not mean that no virus was destroyed.

Variations in injection technique.—Two experiments were performed to compare the survival and infectivity of *A. constricta* nymphs injected in the head, thorax or abdomen. Insects were injected in turn in each of the 3 positions so that when a capillary glass needle broke, the number of insects injected in each position by that needle was the same or differed by not more than one. Insects were tested singly for infectivity. The number of survivors and their infectivity was approximately the same for insects injected in the thorax (29 of 51 survived; 20 were infective) as for those injected in the abdomen (36 of 51 survived; 20 were infective). A smaller number of those injected in the head survived (16 of 51) and their infectivity (6 of 16) was less than that of those injected in the other 2 positions. However, the difference was small and may not be significant. In 2 experiments injections in various parts of the abdomen were compared, but no differences in either survival or infectivity were obtained. In other experiments the insects were injected in the abdomen.

Nymphs in their late instars were usually chosen for injection, but in some experiments younger ones were injected with equally satisfactory results.

RESULTS.—*Recovery from different hosts.*—The wound-tumor virus has been successfully recovered from extracts of *A. constricta*, *A. novella*, *T. incarnatum*, and *Melilotus alba* Desr. (in the latter instance from stem tumors and from portions of stems free of tumors).

Dilution end-point.—Table 1 shows the infectivity obtained from the injection of different dilutions of extracts from *T. incarnatum* and *A. constricta*. Most of the data reported in the leafhopper series are from the serial transmission experiments (8). Infections were obtained with dilutions as high as 10^{-4} from crimson clover extracts and 10^{-5} from insect extracts.

Longevity in vitro.—Two extracts were tested for survival of the virus when stored in cell-free preparations under various conditions. One extract was a $10^{-1.5}$ dilution from 80 viruliferous *A. constricta* insects and 140 viruliferous *A. novella* leafhoppers. The second was a concentrate prepared by 2 cycles of differential centrifugation from diseased crimson clover with a final dilution of 6:1 (10 ml. of concentrate from 60 gm. of plant tissue). Both extracts were in solvent 3. Several samples from each extract were stored in 4 different ways: A. at 0°C. in an ice bath; B. dried in a vacuum desiccator over $CaCl_2$ at 0°C. and stored under the same conditions; C. frozen in a dry-ice acetone bath and stored at −25°C; and D. frozen in a dry-ice acetone bath followed by lyophilization and storage at −25°C. After the desired periods of time, frozen samples were thawed while dry samples were suspended in water to bring them to their original volume.

Table 2 shows the infectivity of insects injected with these samples. No insects survived which were injected with samples stored in solution at 0°C. for 8 days or longer. Five months after the experiment started the cold room was not working for a period of 4 days during which time the dried samples stored at 0°C. were actually at 20°C. Despite this change they did not lose their infectivity. The extract from insects showed greater infectivity and longer survival than that from plants. This may have been due to a higher initial concentration since in other experiments concentrates prepared by differential centrifugation from crimson clover extracts often were less infective than crude extracts, suggesting that considerable virus was lost during such attempted purification. However, the experiment was started before this knowledge was available. It is clear that the virus may be stored in the desiccated or frozen state for as long as a year and still retain some infectivity.

Thermal inactivation.—Small samples of extracts from viruliferous *A. constricta* insects at a $10^{-1.5}$

TABLE 2.—*Longevity of the wound-tumor virus in vitro*

Storage conditions [a]	Storage Period								
	2 days	4 days	8 days	2 weeks	4 weeks	8 weeks	17 weeks	9 months	12 months
				Virus from leafhoppers					
A. ice bath	5/6[b]	4/6	---[c]	---	---	---	---	---	---
B. dried	2/6	5/6	4/6	3/6	4/6	5/6	5/6	2/6	5/12
C. frozen	4/6	4/6	5/6	6/6	3/6	6/6	2/6	3/6	0/12
D. lyophilized	5/6	4/6	3/6	3/6	4/6	4/6	---	---	---
				Virus from Crimson Clover					
A. ice bath	3/6	2/6	---[c]	---	---	---	---	---	---
B. dried	3/6	2/6	0/6	0/6	0/6	0/6	1/6	0/6	---
C. frozen	4/6	6/6	3/6	5/6	2/6	0/6	0/6	0/6	---
D. lyophilized	1/6	1/6	0/6	0/6	1/6	0/6	---	---	---
Uninjected controls	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/12

[a] See text for details of treatments.
[b] Number of colonies infected in the numerator; number of colonies tested in the denominator. 5 insects injected for each colony.
[c] Insects injected with extracts stored at 0°C. for 8 days or more did not survive.

dilution were heated in a water bath at various temperatures for 10 minutes. The samples were rapidly cooled by immersion in an ice bath immediately after being heated. Altogether, in 3 separate experiments, 10 of 18 colonies were infective after the insects were injected with crude extracts (pH 6.6–6.8) treated at 50°C. for 10 minutes. None of 18 colonies were infective after injection of the insects with corresponding extracts treated for 10 minutes at 60°C.

Stability at various pH's.—Several experiments were made to determine the stability of the virus at various pH's at 0°C. Different buffers were used to prevent the possibility of an effect due to some other ion being mistaken for one due to the hydrogen ion. The phthalate-phosphate-borate buffer of Best and Samuel (1) and citrate-phosphate buffers were used. In some experiments with unpurified plant extracts no buffer was added, the pH being adjusted by addition of 0.1 N NaOH or HCl. In 1 experiment the samples were stored at various pH's for 23 hours; in all other experiments the storage time was 1 hour. All pH values were measured on a Beckman Model G pH meter with sodium ion corrections being made at high pH's. Adjustments of pH, both to the storage pH and then back to pH 7.0 for injection, were made on the basis of test titrations on aliquots at room temperature.

Some virus survived storage at 0°C. for 1 hour between pH's 4 and 9 (Table 3). The partially purified crimson clover preparations stored at pH 4 were significantly less infective than those stored at pH 7. These partially purified preparations also gave a lower percentage of infective colonies when injected after storage at pH 7 than the other preparations used for experiments in Table 3. It is probable that the attempted purification resulted in loss of virus. The concentration of virus in these partially purified preparations was small enough so that after storage for an hour at pH 4 there was a barely detectable amount left. With extracts containing a higher original concentration of virus there was still enough left after an hour at pH4 to give a high percentage of infective colonies of injected insects.

TABLE 3.—*Survival of the wound-tumor virus after storage for 1 hour at 0°C. at various pH's*

Total Number of Experiments	pH of storage										Uninjected controls
	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	
1[a]	---	---	4/5[e]	---	3/6	---	5/7[f]	---	---	---	0/4
3[b]	0/18	1/18	---	---	8/18	---	---	0/6	0/18	0/18	0/18
3[c]	0/6	6/6	---	---	5/6	5/6	2/12	0/18	0/18	0/12	0/18
1[d]	0/6	1/6[g]	4/6[h]	4/6	5/6	---	---	---	---	---	0/6

[a] Viruliferous *A. constricta* macerated directly in Best and Samuel's (1) buffer.
[b] Extract from crimson clover partially purified by 1 or 2 cycles of differential centrifugation. Best and Samuel's buffer used for 1 experiment and citrate-phosphate buffers for the other two.
[c] Unpurified extracts from crimson clover at dilutions of 1:2, 1:3 and 1:4 in solvent 3. pH adjusted by addition of 0.1N NaOH and 0.1N citric acid in 2 experiments and by addition of 0.1N NaOH and 0.1N HCl in the third.
[d] Extract from crimson clover partially purified by 1 cycle of differential centrifugation and stored for 23 hours at 0°C. in 0.125 M NaCl and McIlvaine's citrate-phosphate buffer at half its original concentration.
[e] Number of colonies infective in the numerator; number of colonies tested in the denominator. Five insects per colony, 6 colonies at each pH in most experiments.
[f] Actual pH 9.3.
[g] Actual pH 4.2.
[h] Actual pH 5.4. All other pH's were within 0.1 of a unit of the listed pH.

Passage through bacteria-retaining filters.—The virus was passed successfully through Berkefeld and Jenkins filters. One-tenth ml. of a 24-hour culture of *Serratia marcescens* Bizio was added to 10 ml. of an extract from *A. constricta* insects diluted 1:23 in 0.25 M NaCl; part was then filtered through a Berkefeld N filter and another part through a W filter. Nutrient broth was passed through the filters immediately before the virus solution. The extract was tested for virus and bacteria both before and after filtration. One loopful of the unfiltered extract was inoculated into each of 2 test tubes of nutrient-dextrose broth. Another loopful was mixed with nutrient-dextrose agar when the agar was poured into each of 2 petri plates. The filtrate was tested in the same manner except that 0.1 ml. was used for inoculum instead of a loopful. Those plates and tubes inoculated with the unfiltered extract always showed abundant growth within 24 hours. The broth inoculated with the Berkefeld W filtrate showed no growth after 2 weeks; the plates had no growth after 1 day but after 2 weeks 1 plate had 1 bacterial colony. The broth inoculated with the Berkefeld N filtrate showed a whitish growth within 2 weeks; the 2 plates showed a total of 7 colonies at the end of 2 weeks.

An extract from diseased crimson clover in solvent 3 at a dilution of 1:2.5 was filtered through the Jenkins filter. The pH was adjusted to 7.3 with K_2HPO_4 and NaOH. Twenty ml. of nutrient-dextrose broth at pH 7.6 was filtered first, followed by 10 ml. of the virus extract to which 0.1 ml. of a 24-hour culture of *S. marcescens* had been added. The same tests for bacteria were run as in the experiment with the Berkefeld filters. Plates and tubes inoculated with the unfiltered extract showed abundant growth in 24 hours; those inoculated with the filtrate from the Jenkins filter showed no growth in 5 days. Each filtrate was injected into 60 insects and the survivors tested in colonies of 5. Twenty of 29 colonies produced infections; none of the 120 uninjected control insects did so.

Discussion.—The pH stability, thermal inactivation and longevity *in vitro* experiments indicate that the wound-tumor virus is not so unstable as some plant viruses which can be readily transmitted mechanically from plant to plant. It appears to be as stable as the potato yellow-dwarf virus (*Aureogenus vastans* (H) Black) and more stable than the tomato spotted-wilt virus (*Lethum australiense* Holmes) and yet both of these give good local lesions. The inability of the wound-tumor virus to be transmitted mechanically from plant to plant is probably not due to its instability.

SUMMARY

Injection of leafhopper vectors was used as the basis of a bioassay for the determination of certain properties of the wound-tumor virus (*Aureogenus magnivena*). Extracts from crimson clover (*Trifolium incarnatum*) were infective at a dilution of 10^{-4}; those from *Agallia constricta* at a dilution of 10^{-5}. The virus when dried at 0°C. survived storage for 12 months at 0°C. Crude, neutral extracts retained infectivity after being heated at 50°C. for 10 minutes but not after 10 minutes at 60° C. Extracts were infectious when stored at pH's between 4 and 9 for an hour at 0°C. but not when stored under more alkaline or more acid conditions. The virus was successfully passed through filters that retained *Serratia marcescens*.

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SMALL GRAIN SEED TREATMENT WITH THE ANTIBIOTIC, HELIXIN B[1]

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Antibiotics are of much theoretical interest and potential practical importance in plant pathology. During their relatively brief history in this field, they have been used in several ways, including the treatment of seed. The first report of the use of an antibiotic as a seed treatment material was that of Brian and Hemming, who in 1945 showed that gliotoxin had fungitoxic activity in tests with 3 small grain diseases (4). Since then there have been a number of reports dealing with treatments of seed or tubers in greenhouse or field tests (1, 2, 3, 5, 6, 7, 8, 9, 15, 17, 18, 19, 20). These papers record a number of instances of effective disease control with antibiotics, but as yet these materials have not come into general use for treating seed.

The present work describes small grain seed treatment tests in the greenhouse and in the field using the antibiotic helixin B. A short account of some of this work has already appeared (10). The helixin antibiotics are produced by a *Streptomyces* sp. that was obtained from soil. This organism was selected for intensive study on the basis of screening tests designed to discover antibiotics inhibitory to plant pathogens (16). In *in vitro* experiments the partly purified antibiotic inhibited all fungi tested (12), and in greenhouse tests the material was shown to act as a protectant fungicide at low concentrations (11). Partly purified helixin was demonstrated later to contain at least 4 antibiotics, which were separated and named helixins A, B, C, and D (14). Of the 4, helixin B is produced in greatest amounts in tank cultures.

MATERIALS AND METHODS.—*Treating techniques.*—Since helixin B has not yet been obtained in the pure form, partly purified material was employed in these tests. Preparation No. 20 was used in the seed treatment work; this material, which was made as previously described (14), contained 1.8 units per mg. In this state of purity helixin B is water insoluble but is soluble in ethanol, which was used as the solvent for the present work. Early studies showed that ethanol had deleterious effects on the germination of seed. About a 25 per cent reduction of emergence was noted when 0.8 ml. of ethanol was mixed with 5 gm. of oat seed in a 125 ml. Erlenmeyer flask and the seed allowed to remain in the unstoppered flask overnight. If the seed was spread out and the ethanol allowed to evaporate soon after treatment, however, no inhibitory effects were noted. With the exception of 1 test, No. 1 of Table 1, this rapid drying practice was followed in all of the experiments that will be reported. An ethanol control was included in most of the tests, and in all tests emergence data were taken. There was no evidence that ethanol or helixin B in ethanol inhibited emergence or subsequent plant growth if the seed was dried rapidly. The ethanol treatment alone did not lessen the incidence of the various diseases.

Seed was treated in 125 or 250 ml. Erlenmeyer flasks; seed lots varied from 5 to 30 gm. In most tests treated seed was dried and replaced in the treating flask, which was stoppered for at least 4 days before seed was planted. Ceresan M (7.7 per cent ethyl mercury *p*-toluene sulfonanilide) was used for comparative purposes in most of the tests; seed treated with this material was also stored at least 4 days in a closed flask before planting.

Certain Helminthosporium blights of oats and barley.—In the present work the chief emphasis was placed on determining the effects of a helixin B seed treatment on the control of the *Helminthosporium* seedling blights of Victland oats and Wisconsin Barbless barley, which are incited by *H. victoriae* M. and M. and *H. sativum* P. K. and B., respectively. Naturally infested seed was used. Tests were made in the greenhouse and in the field. Of the 2 seedling blights, we have found that the 1 incited by *H. victoriae* has been the least tractable to seed treatment and for that reason seemed especially suitable for testing a new fungicide.

In greenhouse tests there were 4 replicates of 50 seeds for each treatment. Plants were grown in flats at 22–24° C. In experiments with *H. victoriae* the soil was kept relatively dry, a condition that appeared to increase the incidence of the disease. Seedling emergence data were taken 7–10 days after planting, and the numbers of diseased seedlings recorded at 19–21 days. Computation of the percentage of disease was based on the sums of the numbers of diseased plants and emerged plants in all 4 replicates.

Plants were considered diseased with *H. victoriae* if they were withered or if characteristic bronze-colored leaf areas were evident. The criterion for disease with *H. sativum* in greenhouse tests was the browning of the coleoptile and crown region of the seedling, which was observed after the soil had been washed from the roots. The leaves and culms of plants with intense browning were usually stunted or withered. In the field only stunted and withered plants were recorded as diseased, since plants were not removed from the soil for observation.

RESULTS.—*Helminthosporium blight of Victland oats.*—The results of 4 greenhouse tests with this disease are given in Table 1. Rapid drying of seed after treatment was not followed in test 1, and there was ap-

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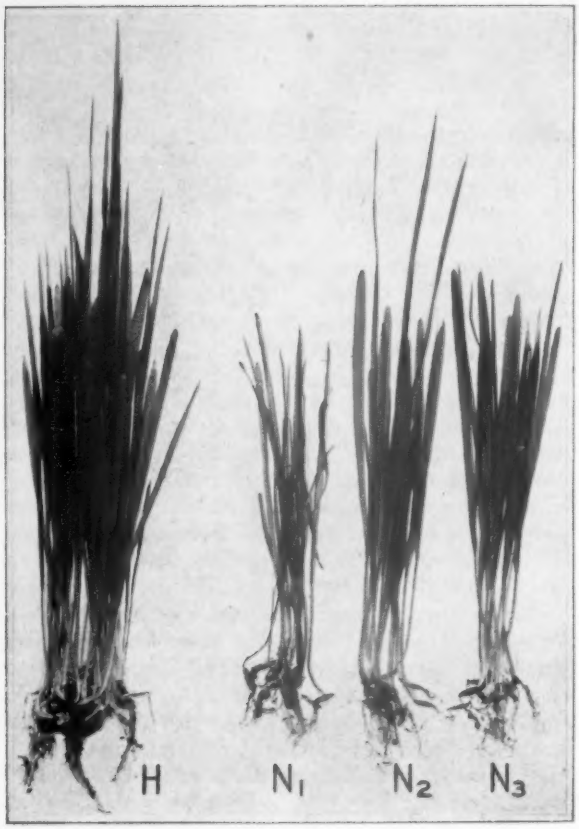

FIG. 1. The control of the *Helminthosporium* blight of Victand oats using helixin B as a seed treatment material —representative replicates from a greenhouse test. Replicate H: seed treated with helixin B (no disease). Replicate N: seed not treated (plants divided into disease classes. N_1, plants severely diseased; N_2 plants moderately diseased; N_3, healthy plants). Note absence of discoloration on the seed and culms of plants from helixin B-treated seed.

proximately a 25 per cent reduction in emergence. In all 4 tests, disease control afforded by the helixin B treatments compared favorably with that obtained with Ceresan M. An example of the effect of the helixin B treatment is shown in Figure 1.

TABLE 1.—*The effect of treating Victand oat seed with helixin B on the incidence of the H. victoriae seedling blight. Greenhouse tests*

Fungicide	Mg. per gm. of seed	Per cent seedling blight			
		Test 1	Test 2	Test 3	Test 4
Helixin B[a]	4.5	0	<1	0	—
Do./	2.3	0	2	3	—
Do./	1.1	<1	0	6	—
Do./	0.6	<1	2	5	—
Do./	0.3	—	6	1	4
Ceresan M	0.9[b]	7	4	3	4
None	—	39	28	39	33

[a] Applied in ethanol at the rate of 0.16 ml. per gm. of seed.
[b] Equivalent to 0.5 oz. per bushel.

TABLE 2.—*Helixin B as a seed treatment material for the control of the H. victoriae disease of Victand oats. Field test 1*

Fungicide	Mg. per gm. of seed	Per cent seedling blight	Av. yield of grain in gm. per row[c]
Helixin B	2.7[a]	<0.1	123
Ceresan M	0.9[b]	0	131
None	—	2	90

[a] Applied in ethanol at the rate of 0.1 ml. per gm. of seed.
[b] Equivalent to 0.5 oz. per bushel.
[c] Yield taken from 2.4 m. of 3 m. rows. L.S.D. at 5 per cent point = 35.3 gm. Yield on basis of bushel per acre: helixin B, 46.1; Ceresan M, 49.1; none 33.8.

In 2 greenhouse tests with this disease, infested seed was treated with finely ground helixin B dust instead of an ethanol solution of the antibiotic. In the first test the concentrations of the antibiotic were 1.1, 2.2, and 4.4 mg. per gm. of seed, and in the second the concentration was 2.4 mg. per gm. of seed. In both tests seed was stored in stoppered flasks for 4 days before planting. The dust treatments gave no control of the seedling blight in either experiment. The disease was controlled, however, by the antibiotic in ethanol and by Ceresan M in tests made at the same time.

Three field tests were made at Madison, Wisconsin, in 1952. In test 1 there were 3 replicates for each treatment, and each replicate consisted of 10 gm. of seed per 3 meter row. Plantings were made April 29. Data for this test are given in Table 2. They indicate that the helixin B treatment reduced the seedling phase of the disease. The yield increase over the control approached significance; there was no significant difference in yield between the helixin B and the Ceresan M treatments. At harvest time the plants in the helixin B or Ceresan M replicates were clearly larger and more vigorous than those in the control replicates.

Field tests 2 and 3 were made during the last of May and the middle of June, well past the usual planting time. Each treatment consisted of 3 replicates of 100 seeds per 1 m. row. Helixin B was used at rates of 0.15, 0.3, 0.6, and 1.2 mg. per gm. of seed; all concentrations were applied in ethanol at a rate of 0.04 ml. per gm. of seed. In both tests the control of the seedling blight afforded by helixin B was far less than that given by Ceresan M. The reduced control is thought to be due to inadequate coverage of the seed with the small amount of solvent that was used. More work will be necessary to ascertain this point.

H. sativum seedling blight of barley.—The results from 3 greenhouse tests with this disease are given in Table 3. Three field tests were made at the same time and in the same manner as the field tests with oats (see preceding section). Data are in Table 4. In all of the experiments with *H. sativum*, helixin B compared favorably with Ceresan M as an effective agent for the control of the disease.

Seed treatment with helixin B for other diseases.—

TABLE 3.—*Helixin B as a seed treatment material for the control of the H. sativum seedling blight of barley. Greenhouse tests*

Test	Fungicide	Mg. per gm. of seed	Per cent diseased plants
1	Helixin B[a]	1.8	1
	None	—	72
2	Helixin B[a]	1.8	5
	Do.	0.9	5
	Do.	0.45	14
	Do.	0.22	17
	Ceresan M	1.2[b]	13
	None	—	92
3	Helixin B[a]	1.2	2
	Do.	0.6	5
	Do.	0.3	8
	Do.	0.15	9
	Ceresan M	0.6[c]	3
	None	—	67

[a] Applied in ethanol at the rate of 0.07 ml. per gm. of seed in tests 1 and 2, and at the rate of 0.04 ml. per gm. of seed in test 3.
[b] Equivalent to 1 oz. per bushel.
[c] Equivalent to 0.5 oz. per bushel.

Limited trials were made in the field on the effectiveness of helixin B for control of wheat bunt [*Tilletia foetida* (Wallr.) Liro], oat smut [*Ustilago avenae* (Pers.) Rostr. and *U. kolleri* Wille], covered smut of barley [*U. hordei* (Pers.) Lagerh.], and loose smut of barley [*U. nuda* (Jens.) *Rostr.*]. Plantings were made at the same time and manner as described for field test 1 with the *Helminthosporium* blight of Victland oats. With the exception of the wheat seed, which was heavily dusted with *T. foetida* chlamydospores prior to treatment, the various lots of seed carried the specified disease organisms naturally. Data for 3 of the diseases are in Table 5. With each, the helixin B treatment compared favorably with Ceresan M. In 2 additional tests with barley seed infected with *U. nuda*, no control by helixin B was obtained when the seed was subjected to the usual ethanol treatment followed by rapid drying.

Action across an air space.—We have found that the only materials that have previously proved effective as seed dressings for the *Helminthosporium* blights of oats and barley were certain of the organic mercury compounds. These materials are thought to owe their success, at least in part, to their volatility, although vapor action in controlling disease has been demonstrated for only a few compounds (13). It was of interest, therefore, to determine if partly purified helixin B possessed the property of acting across an air space. Tests were made with depression microscope slides (depression volume 0.46 cc.). Four helixin B preparations were used: No. 20 and 3 others, which contained 0.9, 2.6, and 3.1 units per mg., respectively. Five mg. of the test material was placed, dry, in the depression. A cover slip on the under side of which was suspended a drop of water containing spores of *Glomerella cingulata* (Stonem.) Spauld. and v. Schrenk was placed over the depression. The slide and the slip were sealed with petrolatum. There were control slides containing no antibiotic in each test. In 18 experiments the germination of spores was prevented or very greatly retarded by the helixin B preparations. The inhibition did not appear to be due to the absorption of gases necessary for spore germination, since germination was likewise inhibited when 1 side of the cover slip was elevated 2 mm. from the slide and the whole assembly placed in a Petri dish containing water.

TABLE 4.—*Helixin B as a seed treatment material for the control of the H. sativum seedling blight of barley. Field tests*

Fungicide	Mg. per gm. of seed	Per cent seedling blight		
		Test 1	Test 2	Test 3
Helixin B[a]	2.3	0	—	—
Do.	0.6	—	1	2
Do.	0.3	—	3	2
Do.	0.15	—	3	4
Do.	0.07	—	6	6
Ceresan M	0.6[b]	0	2	5
None	—	10	18	17

[a] Applied in ethanol at the rate of 0.8 ml. per gm. of seed in test 1 and 0.4 ml. per gm. of seed in tests 2 and 3.
[b] Equivalent to 0.5 oz. per bushel.

TABLE 5.—*The effect of treating small grain seed with helixin B on the incidence of certain seed-borne diseases. Field tests*

Disease	Plant variety[a]	Mg. per gm. of seed		Per cent smutted heads with:		
		Helixin B[b]	Ceresan M[c]	Helixin B	Ceresan M	No fungicide
Wheat bunt	Sturgeon	1.8	0.5	0.1	0.6	3
Oat smut	States Pride	1.8	0.9	0.1	0	5
Do.	Canadian	2.7	—	0	—	17
Do.	Ajax	2.7	0.9	0	0	1
Covered smut of Barley	Moore	2.2	0.6	0	0	6
Do.	Do.	2.2	0.6	0	0	2
Do.	Do.	2.2	0.6	0	0	3

[a] All tests made at the same time. The 3 lots of Moore seed were from different sources.
[b] Applied in ethanol at the rate of 0.07-0.08 ml. per gm. of seed.
[c] Equivalent to 0.5 oz. per bushel.

In early work, action across an air space was believed due to helixin B (10), but 2 subsequent attempts to obtain the active material by molecular distillation failed. The inhibition did not appear to be due to contamination of the test material with the n-butanol used in purification (14), since vapor action was observed after repeated washings with petroleum ether followed by a 6 hour period of drying at 0.05 mm. Hg. Likewise, a butanol contamination did not appear to be responsible for the control of the *H. victoriae* blight, since when butanol was substituted for helixin B in the ethanol used to treat infested seed and the seed planted in the greenhouse in the usual manner, there was no reduction of disease; parallel tests with Ceresan M and helixin B in ethanol gave good control. The importance of the vapor action of the helixin B preparations in disease control is unknown. Also there is not enough information to ascertain what substance is active in this manner, since the preparations used in these studies were not pure. If the active material proves to be helixin B the antibiotic is unique, since to our knowledge no antibiotic of microbial origin has been shown to have this property.

Discussion.—The present work reports successful greenhouse and field experiments with helixin B as a seed dressing for the control of certain small grain diseases. Although in several tests helixin B compared favorably with a standard material, the investigations thus far completed leave unanswered many questions, including those dealing with the suitability of the solvent and the most suitable concentration of the antibiotic for the control of the various diseases. Why helixin B dusts are not effective is also unknown. It would be of interest to compare the effectiveness of helixin B with helixins A, C, or D, respectively, and with more easily made and less purified preparations containing all 4 helixin antibiotics. Some of these problems are now being investigated. Conclusions regarding possible practical adaptations of the helixins for seed treatment must await the results of further work.

SUMMARY

In small scale greenhouse and field experiments with the *Helminthosporium victoriae* blight of Vicland oats, seed treatments with a partly purified preparation of the antibiotic helixin B resulted in disease control in most tests. In similar experiments with Wisconsin Barbless barley, seed treatments with the antibiotic controlled the seedling blight incited by *H. sativum*. Seed treatment with the antibiotic was effective for controlling wheat bunt, oat smut, and covered smut of barley in limited field tests. Helixin B preparations acted across an air space to inhibit the germination of spores of a test fungus.

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FUSARIA ASSOCIATED WITH CITRUS FEEDER ROOTS IN FLORIDA[1]

C. D. Sherbakoff

In 1945, Suit (2) initiated studies on spreading decline, a disease affecting citrus trees, the symptoms of which are mainly these: a) the foliage is conspicuously thin and fruit production very low, b) the feeder root system is reduced, c) the feeder roots deteriorate shortly after they develop, and d) the disease spreads through the soil.

The symptoms of spreading decline thus pointed definitely to the feeder roots as the seat of the disease and indicated the necessity of a systematic study of the microorganisms associated with feeder roots of the diseased trees. Hence extensive series of isolations of the microorganisms from feeder roots of decline-affected and decline-free citrus trees were made at the Citrus Experiment Station, Lake Alfred, Florida, from 1945 to 1952 by various workers (1, 2, 3, 4). All the isolations gave similar results, especially in the predominance of cultures of different Fusaria among the various fungi isolated from citrus feeder roots.

EXPERIMENTAL WORK.—The writer continued the isolations of various microorganisms from citrus feeder roots for 6 months beginning February 1, 1952, and gave special attention to the study of Fusaria associated with roots of diseased and healthy citrus trees.[2] All the isolations were made from pieces of feeder roots about 1 mm. long. Twenty-five to 36 of the pieces were planted on potato-dextrose agar in a petri dish, with 12 dishes in each test. Some of the roots were previously treated for 10–15 seconds in 1:1000 $HgCl_2$ solution in 50 per cent alcohol; others were thoroughly washed in a continuous flow of tap water, then rinsed in several changes of sterile water; still others were immersed in concentrated H_2SO_4 for about 15 seconds, then washed in sterile water.

There were no significant differences in results obtained by these treatments. The healthy roots from healthy trees, and from decline-affected trees when effectively surface disinfected, gave no growth of any microorganism. However, diseased rootlets, whether they were from decline-free or decline-affected trees, produced the same microorganisms, though often in much different proportions.

Results of these isolations were similar to those of others who listed fungi found. During the first 5 series of isolations, a record was kept of the numbers and kinds of fungi that developed colonies from root pieces planted in petri dishes. The counts showed that out of 1000 colonies about 40 per cent were Penicillia and 45 per cent Fusaria. Other fungi, such as *Trichoderma, Thielavia, Chaetomium, Sclerotium bataticola, Botrytis terrestris, Helminthosporium,* were more or less frequently obtained. For a time *Sclerotium bataticola* appeared in some instances to be conspicuously associated with roots of decline affected trees.

The fungi which were found to be closely associated with diseased feeder roots of decline-affected and decline-free trees appear at present to have no relation to the spreading decline disease of citrus in Florida. This seems to be so because the same fungi were isolated from both decline and healthy trees, because negative results were obtained in inoculation experiments by Bliss[3] and others and by the author with citrus seedlings grown in large test tubes on Czapek's agar without sugar.

FUSARIA OF CITRUS ROOTS.—All cultures studied, whether isolated by the writer or by the others, belonged to either the Martiella or Elegans sections, except 3. One of the exceptions was *F. semitectum;* the other was *F. equisetii* which was isolated twice. The Martiella and Elegans are distinct from all other known Fusaria by easily recognizable characters, typical presence of microconidia and terminal and intercalary chlamydospores (5). However, separation of these 2 sections from each other is sometimes difficult and could be made more certain by the 2 previously unreported characters. One, the difference in conidiophores bearing microconidia (Fig. 1, A and D) and, two, that the content of end-cells of macroconidia of Martiella Fusaria is definitely lighter than that of the inner cells (Fig. 1, C) while all cells of macroconidia of Elegans Fusaria are of an even density.

For nearly all cultural work, potato-dextrose agar was used, made from 100 gms. of potatoes, 10 gms. of dextrose, 20 gms. of agar, 500 cc. of distilled water and 500 cc. of soil extract. The soil extract was prepared by mixing 1 part soil to 4 parts of water by volume, holding this for 24 hours, with occasional stirring, and then filtering. Each of the original cultures was transferred to 3 test tubes of the modified potato-dextrose agar, 1 to remain in the culture room, 1 to be exposed to direct sunlight, and 1 for exposure to indirect light on north side of a building. The cultures kept outdoors were subject to the variations in outside temperature.

The cultures of Fusaria of the Elegans section kept on the north side during the cool months of March and April produced abundant bright rose-colored aerial mycelium while those exposed to direct sunlight

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[2] Collections of root samples and isolations were made with the assistance of J. R. Christie (1950-51), Jean C. Linebaugh (1951-52), and Troy L. Brooks (1951-52).

[3] Dr. D. E. Bliss, Plant Pathologist, Citrus Experiment Station, Riverside, California, on sabbatical leave, Jan. 1, 1951, to June 30, 1951, conducted the isolation work in progress during that time.

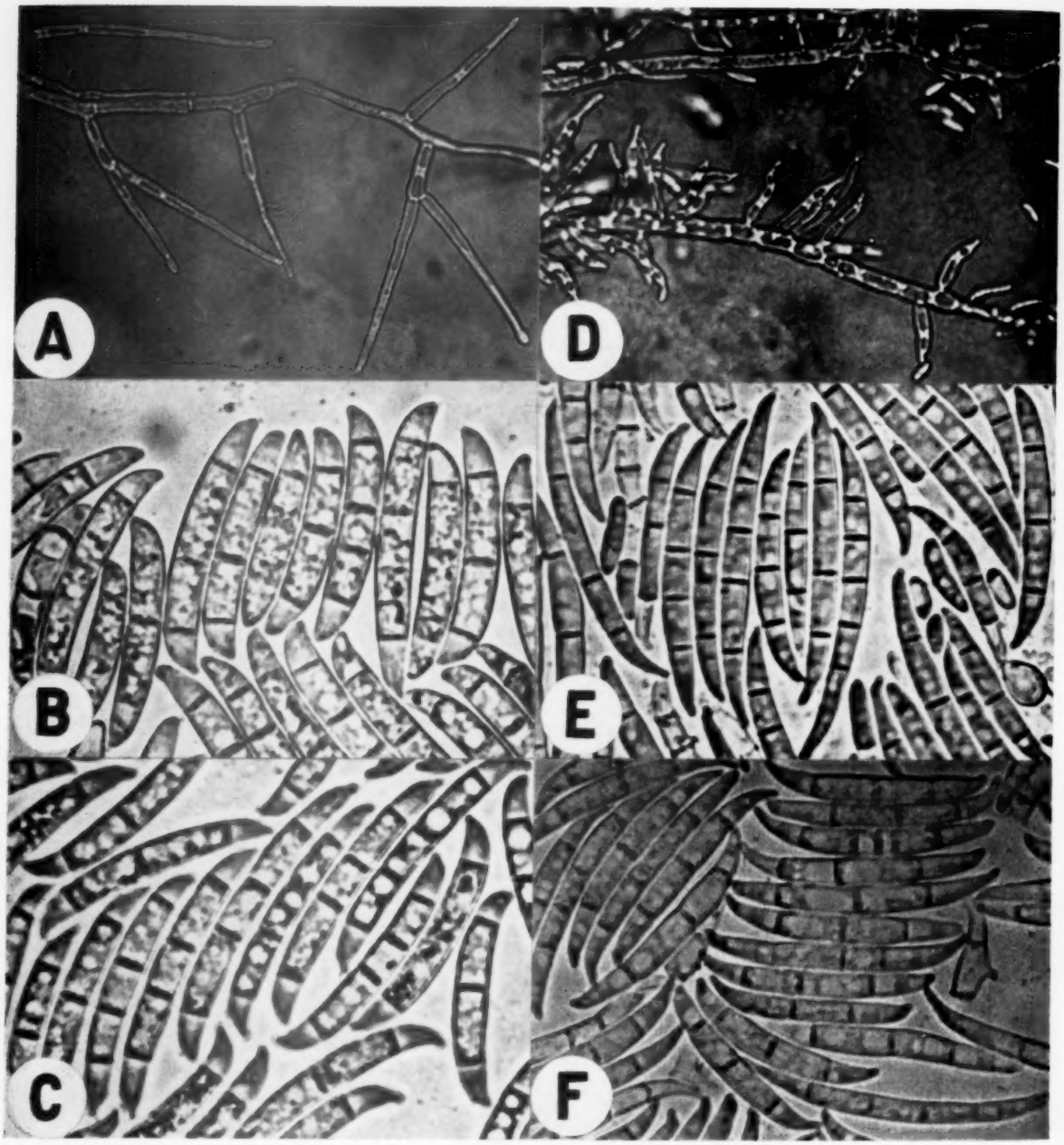

FIG. 1. Fusaria associated with citrus feeder roots in Florida. A) Conidiophores bearing microconidia, typical of section Martiella × 500; B) Sporodochial macroconidia of *Fusarium solani* × 1000; C) Sporodochial macroconidia of *F. solani* var. *martii* × 1000; D) Conidiophores bearing microconidia, typical of section Elegans × 500; E) Sporodochial macroconidia of *Fusarium bulbigenum f.1* × 1000 (Isolate No. 40); F) Sporodochial macroconidia of *F. bulbigenum F.1* × 1000 (Isolate No. 27).

produced scant mycelial growth showing little or no color. Light orange sporodochial macroconidia were produced in greater abundance in direct sunlight than in the shade. During the hot weather and extreme intensity of sunlight in May and June, the culture exposed to direct sunlight in that period produced no normal conidia and some cultures appeared to be entirely arrested in their growth. Nearly all the cultures stored in the culture room were more or less colorless; however, the aerial mycelium of a few developed a bright purple color not observed in the cultures kept outdoors.

All cultures of Fusaria of the Elegans section produced an abundance of sporodochial macroconidia of normal appearance.

Determinations of morphological characters of con-

idia of the different Fusaria were made primarily by means of a series of photomicrographs of characteristic macroconidia illustrating variations found in different cultures.

Out of the entire set of cultures, some were selected for photomicrographing their macroconidia. The selection was made from cultures that were representative of the range of variations in size, shape, and septation of macroconidia observed in all of the cultures. In each of the selected cultures the location of the most uniform and characteristic mass of macroconidia was marked and samples from these were mounted and photographed.

The photographs were taken at 500 × magnification on the negatives and from these contact and 1000 × enlargements were made for measuring the conidia. Usually 10 conidia of each septation group were measured on the 500 × prints with the aid of a reading glass and 20 3-septate conidia were measured on the 1000 × enlargement prints.

The data obtained indicate that by the size of conidia the Fusaria of Martiella section may be placed into 3 groups: Group 1, represented by 6 typical cultures, with conidia of the following size:

3-septate 35 × 5.4 (34-37 × 5.2-5.6) μ
4-septate 41 × 5.4 (38-45 × 5.1-5.6) μ
5-septate 48 × 5.6 (45-52 × 5.4-5.8) μ

These Fusaria may be identified as *F. solani* (Mart.) App. et Wr. Group 2, represented by 8 typical cultures, with conidia.

3-septate 36 × 4.8 (31-44 × 4.6-5.1) μ
4-septate 42 × 5.0 (41-46 × 4.7-5.4) μ
5-septate 49 × 5.1 (44-58 × 5.0-5.2) μ

These cultures may be considered as *F. solani* var. *Martii* (App. et Wr.) Wr. et Rg.

Group 3, represented by 7 typical cultures, with conidia.

3-septate 33 × 5.0 (24-38 × 4.2-5.5) μ
4-septate 39 × 5.1 (36-45 × 5.0-5.2) μ
5-septate 46 × 5.2 (44-48 × 5.0-5.6) μ

These Fusaria, intermediate between those of group 1 and 2, are nearer to group 2 and therefore should be considered also as *F. solani* var. *Martii*.

Of the Elegans section 4 representative cultures of one group were with the conidia as follows:

3-septate 35 × 4.4 (32-36 × 4.3-4.5) μ
4-septate 38 × 4.4 (30-40 × 4.4-4.5) μ, small number recorded
5-septate 40 × 4.3 (38-42 × 4.1-4.4) μ, small number recorded

These cultures, by the size and shape of macroconidia, could be, if to follow Wollenweber and Reinking's classification (5), considered as *F. oxysporum* Schl. amend. Wr.

Another group of Elegans Fusaria was represented by a small number of cultures with rather small macroconidia. These, studied in 2 representative isolations, had conidia as follows:

3-septate 34 × 3.5 (32-34 × 3-4.0) μ
4-septate 40 × 3.6 (38-43 × 3.4-3.8) μ, small number recorded
5-septate 40 × 3.6 μ

These cultures may be identified as a variation of *F. bulbigenum* Cke. et Mass., with smaller size of macroconidia.

In the majority of cultures of the Elegans section the conidia, in 11 typical cultures studied, were of the following size:

3-septate 38 × 3.9 (33-41 × 3.7-4.1) μ
4-septate 42 × 4.0 (38-44 × 3.7-4.2) μ
5-septate 47 × 4.1 (40-58 × 3.7-4.3) μ

These cultures should be identified as *F. bulbigenum* Cke. et Mass. and, because of their common and apparently significant association with citrus feeder roots, are considered to be of a new form of *F. bulbigenum* described here as follows:

F. bulbigenum Cke. et Mass. *forma 1*; aerial mycelium rose colored; macroconidia in orange colored sporodochia over white cushions mostly ½–1 mm. in diameter; macroconidia more gradually attenuated toward the apex than those of other known species of Elegans section. The size of macroconidia in all of the isolates studied is as follows: 3-septate 38 × 3.9 (33-41 × 3.7-4.1) μ; 4-septate 42 × 4.0 (38-44 × 3.7-4.2) μ; 5-septate 47 × 4.1 (40-58 × 3.7-4.3) μ. This Fusarium is usually closely associated with citrus feeder roots in Florida.

SUMMARY AND CONCLUSIONS

The association of Fusaria with citrus roots indicates a probability that they may play some part, not clear at present, in the life of the citrus trees in Florida.

The Fusaria of the Martiella and Elegans sections were found to be closely associated with citrus feeder roots in Florida but they appeared to have no direct pathogenic role in the spreading decline disease of citrus trees.

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PATHOGENICITY STUDIES WITH INBRED LINES OF PHYSIOLOGIC RACES OF TILLETIA CARIES [1]

C. S. Holton [2]

Pathogenicity in physiologic races of the bunt fungi is a heritable character which may be controlled by 1 or more genetic factors (3, 4). Accurate identification and systematic classification of races of these species depend on consistency of reactions in succeeding tests. This in turn largely depends upon degree of genetic purity in races that may be approached by the process of host selectivity (5). The limitations inherent in this process are self-evident, and practical experience has demonstrated that stability of pathogenicity in races may or may not be achieved in this manner (8). A more certain approach to pathogenic homozygosity would seem to be through inbreeding of the races. Recent developments and improvements in methods and techniques (6, 7) have made possible the production of inbred lines[3] of several physiologic races of *Tilletia caries* (DC.) Tul. Results of studies on the pathogenicity of some of these lines are reported here.

MATERIALS AND METHODS.—Physiologic races of *Tilletia caries* used in this study were identified and designated by numbers (T-4, T-6, T-8, T-9, T-10, T-12, and T-15) in earlier studies (8, 9, 10). Both narrow and wide host ranges are represented among these races, and some of them have been stable while others have been unstable in pathogenicity (8). Inoculum has been perpetuated by annual passage of the races through selected host tester varieties.

Forty-eight inbred lines, ranging from 3 to 13 for each race, were tested. These were obtained from inoculations with paired monosporidial lines by methods already described (6). When insufficient inoculum was produced by the first inbred generation, it was increased on a susceptible spring wheat variety. Each inbred line was compared in pathogenicity with the original race. Some of the lines were tested 3 years while others were tested either 1 or 2 years.

The host tester varieties were the same as those used previously (8, 9, 10), with 2 exceptions: Orin (Orfed X Elgin-3) was substituted for Oro, and Sarheim (Hussar X Hohenheimer, C. I. 10068-1) was added to the list. Difference in pathogenicity was measured in terms of smut percentages. These were determined by counting total and smutted heads of wheat in each row.

TABLE 1. *Percentages of smut produced on 4 tester varieties of wheat by 2 races of Tilletia caries and their respective inbred lines*

Race and Inbred Line	Differential Variety and Percentage of Smut			
	Hybrid 128	Orin	Hohenheimer	Sarheim
T-9	84	4	65	2
I. L. 1	83	3	32	2
I. L. 2	83	3	51	1
I. L. 4	80	1	54	2
I. L. 5	65	2	62	3
I. L. 3	75	65	30	46
I. L. 6	47	1	8	0
T-10	78	4	72	2
I. L. 18	66	2	39	0
I. L. 19	83	2	67	1
I. L. 20	69	0	50	0
I. L. 28	38	0	48	0
I. L. 29	53	3	46	0
I. L. 30	86	1	71	4
I. L. 31	86	0	84	0
I. L. 32	89	2	67	1
I. L. 33	83	0	63	1
I. L. 34	82	1	62	1
I. L. 35	82	1	65	0
I. L. 36	93	1	42	0
I. L. 37	76	0	64	0

RESULTS.—Results obtained with races T-9 and T-10 are presented in Table 1. Although their host range is narrow and identical, they differ in several characters (2). Pathogenically their original differentiation was based on the intermediate and high virulence of T-9 and T-10, respectively, on Hohenheimer (2, 9). It is evident from the data in Table 1, however, that this distinction no longer prevails, since both races are about equally virulent on Hohenheimer. This is perhaps explainable by the behavior of inbred lines.

Six inbred lines of T-9 exhibited 4 types of pathogenicity (Table 1). One of these (I. L. 1) was like the original T-9 reaction and another (I. L. 2, 4, 5) was like the current T-9 reaction type. The third reaction type (I. L. 3) was like T-16 (10) and the fourth (I. L. 6) appeared different from any other known race (9). Apparently, therefore, the original T-9 was heterozygous for pathogenicity, which probably explains its pathogenic instability in routine maintenance and testing procedures.

By contrast, T-10 appears to be homozygous (Table 1). Thirteen inbred lines from 2 spores exhibited the same general pathogenic properties as the parent

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Cooperative investigations of the U. S. Department of Agriculture, Agricultural Research Administration, Bureau of Plant Industry, Soils, and Agricultural Engineering, Division of Cereal Crops and Diseases and the Washington Agricultural Experiment Station. Scientific Paper No. 1187, Washington Agricultural Experiment Station, Pullman. Project No. 183.

[2] Pathologist, Bureau of Plant Industry, Soils, and Agricultural Engineering, Division of Cereal Crops and Diseases, Pullman, Washington.

[3] As used here, inbred line refers to the product of inoculation with a compatible pair of monosporidial lines derived from 1 chlamydospore.

TABLE 2. *Percentages of smut produced on 7 tester varieties of wheat by 2 races of Tilletia caries and their respective inbred lines*

Race and Inbred Line	Differential Variety and Percentage of Smut						
	Hybrid 128	Albit	Hohenheimer	Hussar	Martin	White Odessa	Sarheim
T-15	80	89	74	54	64	84	79
I. L. 24	79	90	73	70	78	88	73
I. L. 25	86	95	86	73	83	90	72
I. L. 26	84	93	81	66	85	90	67
T-12	94	78	72	9	28	90	6
I. L. 47	94	86	74	16	51	81	2
I. L. 51	80	65	45	1	38	66	0
I. L. 52	85	66	49	5	28	75	2
I. L. 55	75	82	51	11	55	72	6
I. L. 56	70	65	72	0	10	65	0
I. L. 50	70	60	22	5	18	62	1
I. L. 54	70	56	20	0	24	75	1
I. L. 57	72	40	23	1	14	50	0
I. L. 58	70	46	22	2	5	55	1
I. L. 59	73	71	25	0	4	50	0
I. L. 48	69	60	7	0	5	11	0
I. L. 49	40	28	6	1	0	17	0
I. L. 60	30	13	3	0	1	1	0

race. However, there were slight differences in degree of pathogenicity on certain varieties. For example, I. L. 18 was less virulent on Hohenheimer than T-10 or any of the other inbred lines. In this respect it resembles the orginal T-9 (2, 10). Furthermore, I. L. 28 produced only 38 per cent smut on Hybrid 128. This possibly represents another type of pathogenicity. Further testing is necessary to fully elucidate these tendencies.

Races T-15 and T-12 also exhibit a contrast in pathogenic purity. These races represent wide ranges in pathogenicity, the former being highly virulent on 7 host testers and the latter being more or less virulent on 5 of the tester varieties (Table 2). Three inbred lines (I. L. 24, 25, 26) from 1 spore of T-15 were generally identical with the parent race, indicating homozygosity for pathogenicity in this race. This may not be truly representative, however, since the spore from which the 3 lines were derived produced 10 primary sporidia and had a potential capacity of many more inbred lines. Nevertheless, on the basis of these results T-15 must be regarded as homozygous for pathogenicity. On the other hand, T-12 appears to be heterozygous for pathogenicity.

Thirteen inbred lines from the same spore of T-12 exhibited at least 3 and possibly 4 types of pathogenicity (Table 2). Five of these lines (47, 51, 52, 55 and 56) were essentially like the parent race and 5 others (50, 54, 57, 58 and 59) were similar to T-12 but apparently different in degree of virulence on 2 of the varieties (Hohenheimer and Martin). The remaining 3 lines (48, 49, and 60) were distinctly less virulent than the other 10 on all varieties, with the possible exception of I. L. 48 on Hybrid 128 and Albit.

Representing wide and identical host ranges, T-4 and T-6 were separated originally by a difference in degree of virulence on Albit (10), T-4 being the less virulent. This study shows not only that the difference in degree of virulence on Albit has been lessened but also that both races apparently are heterozygous for pathogenicity (Table 3). At least 3, and possibly 4, pathogenic biotypes were represented in 9 inbred lines from 2 spores of T-4. One of these (I. L. 15) perhaps most nearly resembles the original T-4 reaction (10). Four of the lines (16, 17, 38, and 40) were virtually identical with T-6 by the high virulence on Albit. The other 4 (12, 13, 14, and 48) were distinctly different from either T-4 or T-6 (Table 3). One line (I. L. 48) was especially low in virulence, even on the susceptible variety Hybrid 128.

TABLE 3. *Percentages of smut produced on 4 tester varieties of wheat by races T-4 and T-6 of Tilletia caries and their respective inbred lines*

Race and Inbred Line	Hybrid 128	Albit	Martin	White Odessa
T-4	82	57	72	70
I. L. 15	65	30	65	64
I. L. 16	75	64	73	87
I. L. 17	69	69	70	63
I. L. 38	78	61	42	69
I. L. 40	83	55	36	74
I. L. 12	47	30	48	28
I. L. 13	45	33	55	65
I. L. 14	46	12	67	16
I. L. 48	13	1	2	6
T-6	87	86	76	93
I. L. 43	84	91	78	93
I. L. 44	87	89	62	86
I. L. 46	70	71	67	65
I. L. 45	42	25	6	21

TABLE 4. *Percentages of smut produced on 5 tester varieties of wheat by race T-8 of Tilletia caries and 5 inbreds of this race*

Race or Inbred Line	Hybrid 128	Albit	Hussar	Martin	White Odessa
T-8	70	80	86	70	79
I. L. 9	63	58	46	15	56
I. L. 10	74	57	42	32	68
I. L. 11	64	30	23	20	22
I. L. 7	66	26	11	4	33
I. L. 8	76	1	1	0	3

Four inbred lines of T-6 from 1 spore exhibited 2 types of pathogenicity (Table 3). Three of these (43, 44, and 46) were like the parent race while the other (I. L. 45) was distinctly less virulent on all the host varieties.

Results of studies with T-8 indicate not only a pathogenically heterozygous condition but also a general lessening of virulence in all the inbred lines derived from 1 spore of this race (Table 4). Varying degrees of lower virulence than T-8 were exhibited by all 5 inbred lines on the varieties Albit, Hussar, Martin and White Odessa. The reduction in virulence exhibited by all 5 lines was greatest on Martin. Inbred Line 8 was the least aggressive of the 5 lines, having lost the virulence for all the varieties except Hybrid 128. This reaction is typical of T-1 (10).

SUMMARY AND CONCLUSIONS

Physiologic races of *Tilletia caries* may be either homozygous or heterozygous for pathogenicity. Inbred lines of homozygous races resemble the parent race while those of heterozygous races may exhibit wide ranges in pathogenicity.

A difference in degree of virulence on the same tester variety may not be a valid basis for race differentiation. Such differences may be due to a heterozygous condition of 1 of the races. Differentiation between T-9 and T-10 and between T-4 and T-6 are examples in point.

Host range is not a measure of the homozygous or heterozygous nature of physiologic races. Both these conditions were found in races with narrow and wide ranges in host variety affinity.

Inbred lines of 1 physiologic race (T-8) exhibited generally weaker virulence than the parent race on the same varieties. This suggests the interaction of complementary factors for pathogencity.

The potential problems inherent in the practice of designating pathogenically distinct chlamydospore populations of the bunt fungi as physiologic races have long been recognized (1). The validity of this earlier speculation on the potential instability of pathogenic races of these species is borne out by practical experience (8). This study further emphasizes the complexity of pathogenic specialization in this group of fungi.

The practical significance and implications of these results in relation both to physiologic race studies and to bunt control are clear. Evidently pathogenically homozygous races may or may not be derived from field collections of chlamydospores by passage through a series of host tester varieties. Nevertheless, the general types of virulence existing under field conditions and the basic relationships between them may be established in this manner. Races thus identified can and have been used successfully in studying the genetics of bunt resistance and in developing resistant varieties. However, the pathogenically heterozygous condition of chlamydospore populations in the field unquestionably is the main contributing factor to the dynamics of the problem of specialization in the bunt fungi.

BUREAU OF PLANT INDUSTRY
PULLMAN, WASHINGTON

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PHYTOPATHOLOGICAL NOTES

An Improved Method of Growing Inoculum of Plant Disease Fungi.[1] H. W. SCHROEDER, M. G. BOOSALIS AND M. B. MOORE.—In determining the varietal resistance or susceptibility of crops to disease in the field, large quantities of inoculum for the initiation of an epidemic often are required. The usual method of preparing fungus inoculum in quantity is to grow the fungi on a solid medium in mason jars, milk bottles, or Erlenmeyer flasks. By substituting wooden flats for the smaller containers it has been possible to prepare huge quantities of inoculum with very little effort and a minimum of equipment. Though it might seem impossible to avoid serious losses from contamination by extraneous molds, in actual practice contamination has been negligible. The writers have had success with all the various species of *Fusarium* and *Helminthosporium* tried and it seems probable that many other moderately fast growing fungi could be grown equally well. The fungi thus far tried sporulate more profusely in the flat cultures than when grown in less well aerated bottles or flasks.

[1] To be published as Paper No. 2875 in the Scientific Journal Series of the Minnesota Experiment Station.

Wooden flats about 3 in. deep with hardware cloth bottoms are lined with Kraft paper for use as culture containers (Fig. 1 a). The media used may be varied to conform with the requirements of the various fungi for growth and to the desired method of application of the inoculum. A 2:1 mixture of oats and wheat, or barley and wheat, and a 2:1:2 mixture of oats, wheat and vermiculite have proved satisfactory. The flats are filled ½ full with the media which has been soaked for 24 hours in cold water or 2–3 hours in hot water. They are then covered with Kraft paper (Fig. 1 a) and autoclaved on 2 successive days for 1½ hr. at 15 lb. pressure. When cool, they are ready for inoculation.

Maximum development of the organism with negligible contamination can be obtained by the following method of inoculating the flats: 1) Blend 1 or more Petri dish cultures of the desired fungus with 500 cc of sterile water for about 45 seconds in a Waring blendor. 2) Lift one side of the paper cover and pour the suspension evenly over the medium. The

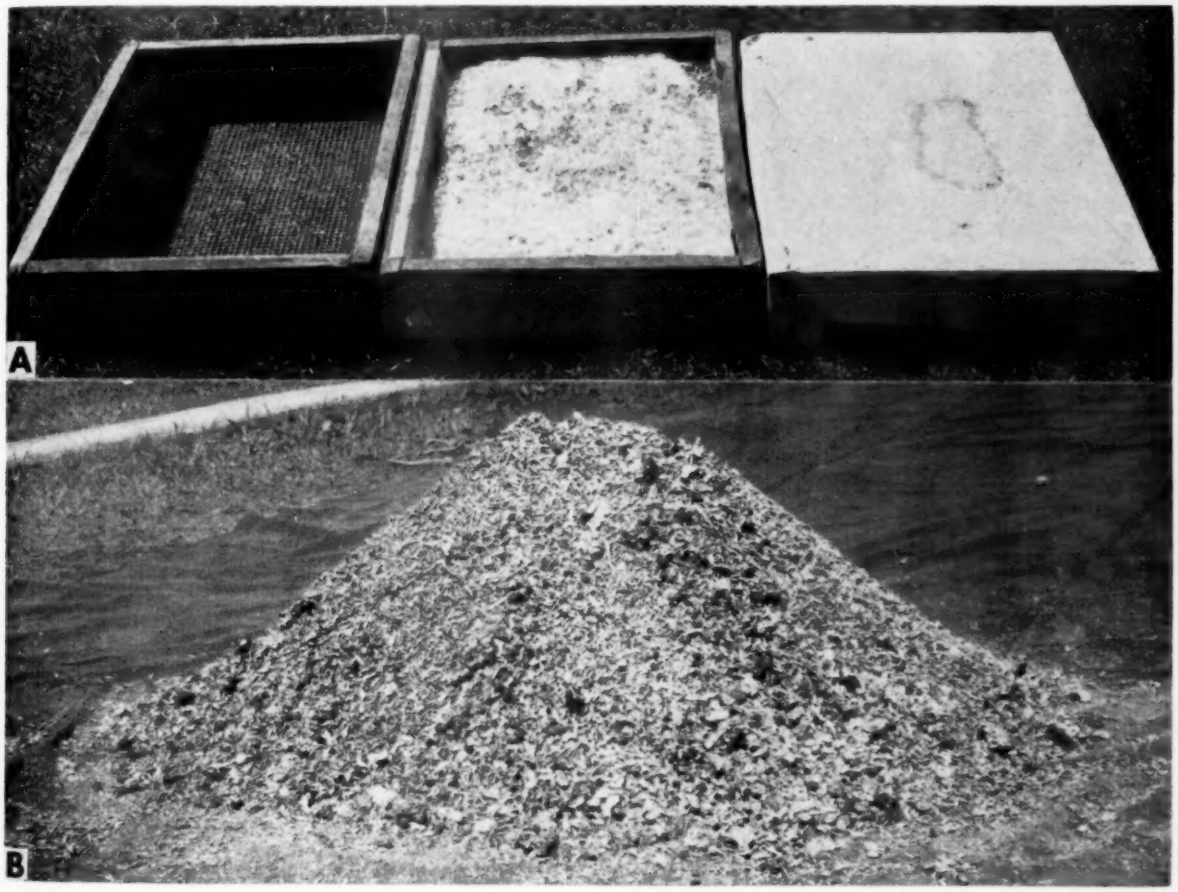

FIG. 1. A) From left to right: empty flat, inoculated flat with cover removed containing a culture of *Fusarium graminearum*, and flat with cover in place. B) Mixed inoculum of *Fusarium* spp. and *Helminthosporium* spp. from 15 flats after drying.

success of the method seems to depend largely upon this heavy and uniform inoculation of the medium and upon the early and rapid growth of the desired fungus which very probably crowds out any contaminants. 3) Replace the cover and fasten it securely with staples or thumb tacks.

The flats may then be stacked at room temperature in a place relatively free from air movements. Sterile spacers should be placed between adjacent flats to allow sufficient aeration. Flats should be exposed or moved as little as possible and all equipment as well as the tables upon which the work is done should be disinfected. In some cases there has been a tendency for the inoculum to heat during growth. This can be reduced by using a shallow layer of inoculum, by adequate aeration, by putting the flats at a lower temperature and, if heating has started, by stirring the inoculum in the flat to loosen the mass and aid in the dissipation of heat. Ten to 14 days has been sufficient for good growth and sporulation of the fungi throughout the flats.

Subsequent handling of the inoculum is determined by the use for which it is intended. For application to soil, the inoculum may be grown several weeks to a month in advance of its intended use. When fully developed it is crumbled and dried, then placed in sacks and stored under refrigeration. The medium containing vermiculite is used for this purpose as it facilitates crumbling the culture mass. When the inoculum is to be applied by spraying, as is done for Fusarial head blight and some of the leaf spotting diseases, the preparation of the flats is timed so that the cultures will have reached maximum development at the time application of the inoculum is required. The suspension for spraying is prepared by washing the media in water then straining the washings through a fine mesh screen. The media may then be poured back into the flats and by placing the flats in a cool shaded place, several successive crops of conidia may be harvested. Conidial formation in some fungi appears to be stimulated by washing the grain media. Vermiculite should not be used in the medium when the inoculum is to be applied by spraying since small pieces of vermiculite pass through the strainer and plug the sprayer.—Division of Plant Pathology, University of Minnesota, University Farm, St. Paul 1, Minnesota.

A Trunk and Crown Rot of Sweet Cherry in British Columbia.[1] D. L. McIntosh. The root crown rot caused by *Phytophthora cactorum* (L. & C.) Schroet. has been widespread on apple trees in the Okanagan Valley, British Columbia, for many years, yet the natural infection of other kinds of fruit trees that have proved susceptible when inoculated artificially, has seldom been encountered. Sweet cherry trees have been found with a rot similar to that produced through artificial inoculation with *P. cactorum*, but attempts to isolate the causal organism hitherto have been unsuccessful. In 1951, however, *P. cactorum* was isolated from a 6-year-old sweet cherry tree with a rot that girdled the trunk from root crown to several inches above ground level. Subsequent surveys of young cherry plantings have disclosed the death of a number of trees with similar symptoms.

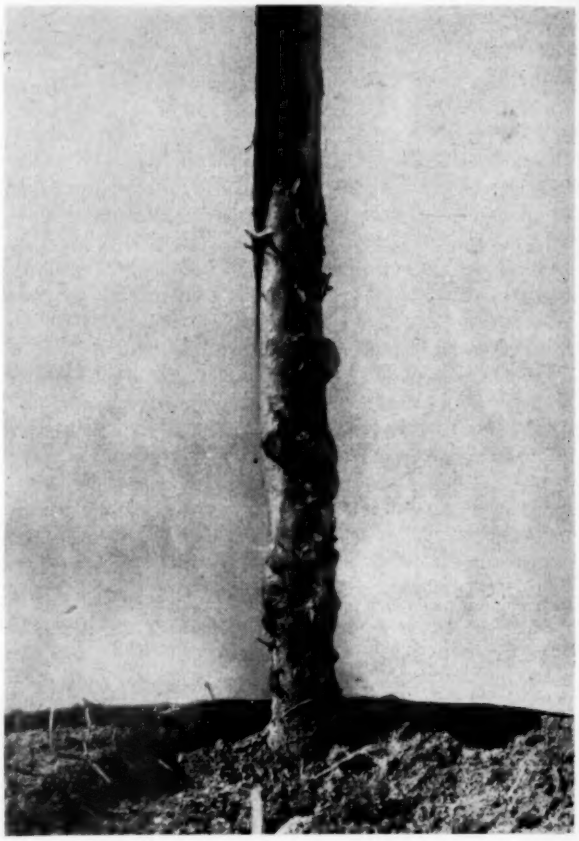

Fig. 1. Gum deposits on trunk of sweet cherry girdled by *Phytophthora cactorum*. (Photograph by S. R. Cannings.)

Unlike the apple crown rot described by Welsh[2] in which killing of tissues occurred for the most part below ground level, the above-ground portion of a cherry trunk is extensively invaded. Gum deposits (Fig. 1) on the surface of the infected area are characteristic and may indeed be the first external evidence of infection. Discoloration of the bark surface can seldom be detected during the season in which infection occurs. Likewise, leaf symptoms are delayed until the disease is well advanced. When girdling of the trunk occurs in spring, leaves may wilt and die in mid-summer; with less severe infections, leaves turn prematurely yellow or red in the fall. When cut into, infected tissue is tan to dark brown in

[1] Contribution No. 1239 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa.

[2] Welsh, M. F. 1942. Studies of crown rot of apple trees. Can. Jour. Res. C 20: 457-490.

color and firm to the knife. Discolored tissue is usually sharply delimited from apparently healthy tissue, but sometimes the area between the two appears water-soaked.

The above-ground portion of the trunk appears particularly susceptible to invasion. When seedling trees were inoculated below ground level, rotting spread rapidly upward, with little downward or lateral development of the lesion.

Observational evidence indicates that an infection may be active for 1 season only. Trees partially girdled in 1 season through artificial inoculation frequently suffered no enlargement of the canker in the following year.—Laboratory of Plant Pathology, Summerland, B.C., Canada.

Aids in the Isolation of Fungi from Woody Tissues. ERNEST E. HUBERT[1] The following equipment has been found useful in making large numbers of isolations from infected wood samples, particularly those showing stages of decay and stain.

The equipment consists of a metal "C" clamp having 3 in. or greater jaw space which is fastened to a board about 5½ in. wide, 15 in. long and ⅞ in. thick, fitted with a metal guard to prevent sliding over the table. The clamp is tilted, raising the jaws above the surface of the board so that the wood sample may be held firmly. Cross pieces on the lower surface of the board elevate it to provide ample space for tightening the jaws.

Blocks of infected wood are cut about 3 in. in length, are washed to remove dust and spores, dipped in boiling water for a moment, and then split open with a sterilized blade to expose 2 inner uncontaminated surfaces. The piece selected for isolations is clamped in the jaws (Fig. 1, D), and slivers or fragments of in-

[1] Research Pathologist, Forest, Wildlife and Range Experiment Station, University of Idaho.

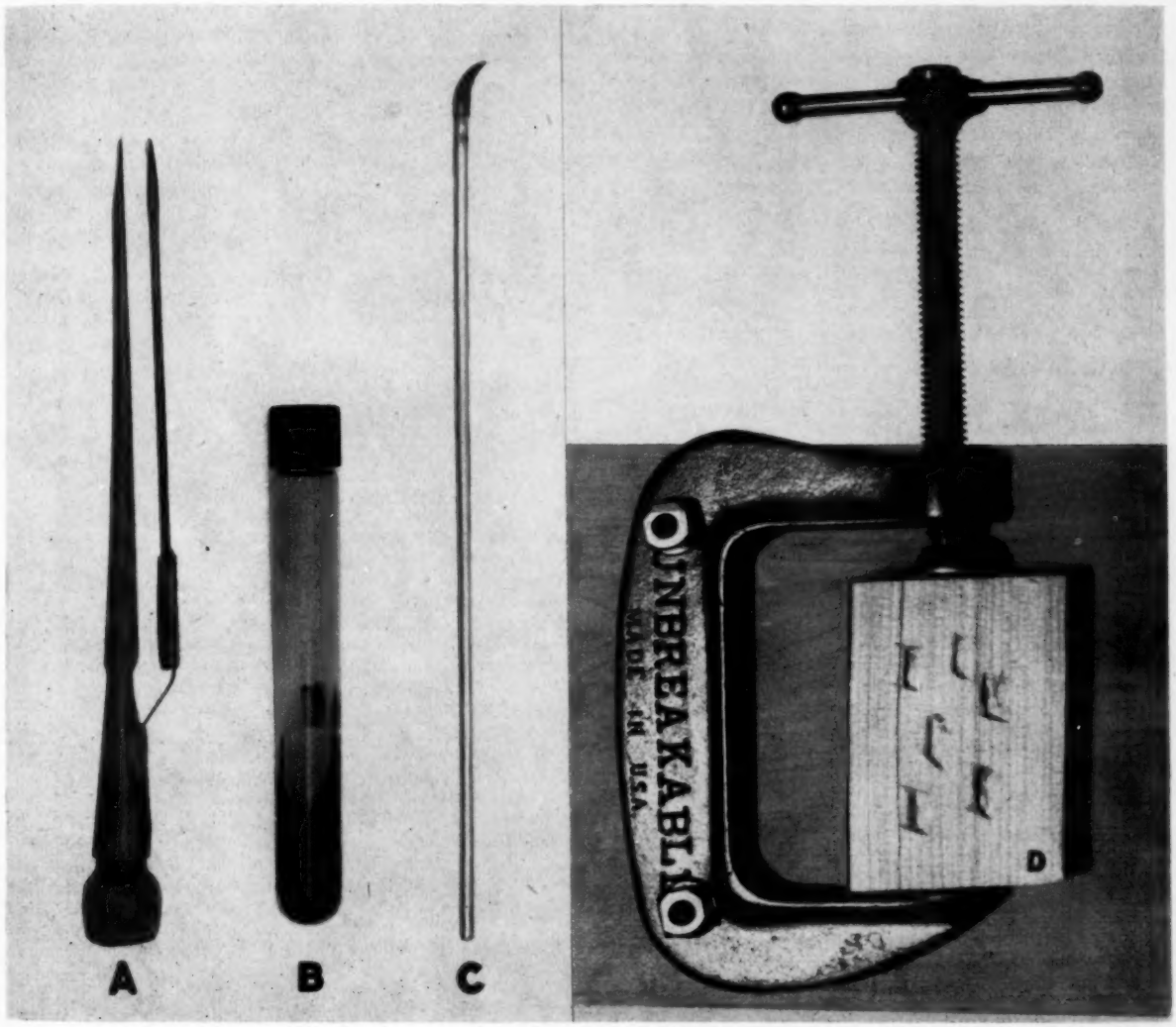

FIG. 1. A) Chisel forceps. B) Culture tube with inoculum fragment imbedded in medium. C) Transfer instrument. D) Infected wood block showing cavities from which fragments were cut and removed. The wood block is held in clamp which is bolted to a board having a metal guard fastened to its lower end.

fected wood are cut by means of a chisel forceps (Fig. 1, A). Two cuts, about ½ in. apart, are made with the sterilized chisel point, usually at an angle with the grain of the wood. The fragment between these cuts is pried up by means of the blade and is then grasped by the forceps and transfered to the culture medium. The fragment can thus be handled rapidly and can be forced half way into the solid medium (malt agar) within the test tube or other culture dish. The transfer instrument (Fig. 1, C) is a No. 12 Bard-Parker scalpel blade fastened to the end of a slender aluminum rod. This instrument has proved useful in cutting the surfaces of "tough" or leathery cultures to remove fragments of fungous tissues for transfer or inoculation purposes. The slender Bard-Parker handle may serve in place of the aluminum rod.

The chisel forceps has been reported [2], but the transfer instrument (Fig. 1, C) has not been reported previously.—University of Idaho, School of Forestry, Moscow, Idaho.

[2] Hubert Ernest E. 1921. A chisel forceps. Phytopathology 11:175.

REPORT AND ABSTRACTS OF THE TENTH ANNUAL MEETING OF THE POTOMAC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The tenth annual meeting of the Potomac Division of the American Phytopathological Society was held in the Plant Industry Auditorium at Beltsville, Maryland, February 19 and 20, 1953. Approximately 80 members attended the sessions, representing the District of Columbia, Delaware, Maryland, and Virginia. Thirty technical papers were presented. The annual banquet was held in the Plant Industry Station cafeteria. Following the banquet Dr. J. G. Harrar spoke on "International Cooperation in Agriculture."

The following officers were elected to serve during the 1954 meeting: *President*, CURTIS MAY; *Vice President*, J. W. HEUBERGER; *Councilor*, W. J. ZAUMEYER.

H. REX THOMAS,
Secretary-Treasurer.

ABSTRACTS OF PAPERS PRESENTED

Tomato Ringspot Virus in the Florists' Hydrangea. BRIERLEY, PHILIP AND FLOYD F. SMITH. *Hydrangea macrophylla* from greenhouses in Pennsylvania in 1947 and in North Carolina in 1952, showing shoot die-back and rugose mosaic, respectively, yielded tomato ringspot virus. The hydrangea virus agrees in symptom production in 15 test species with Price's tomato ringspot virus supplied by R. E. Wilkinson. The virus was manually transmitted from hydrangea in June and from tobacco in July to hydrangea seedlings. No symptoms appeared during 6 months, but the virus was recovered in tobacco from 1 of 10 and from 4 of 5 seedlings, respectively. The hydrangea culture and the type virus manually inoculated into hydrangea seedlings in November each produced chlorotic ringing in 1 of 10 plants in January. These symptoms developed in old leaves that persisted briefly after the plants were returned from storage to greenhouse. Strafford hydrangeas inarch grafted to the rugose mosaic source in June developed yellow mottling in young leaves in January, suggesting the presence of a second virus in this source material. Three species of aphids transferred from the rugose mosaic source and 4 species of aphids and 1 spittle bug from the die-back source have thus far failed to produce any symptom in inoculated hydrangeas.

Experiments with Tomato Aspermy Virus from Chrysanthemum. BRIERLEY, PHILIP, FLOYD F. SMITH, AND S. P. DOOLITTLE. Tomato aspermy virus infected 34 of 73 species tested; was damaging to tomato, pepper, lettuce and spinach; produced primary lesions or no infection in cucumber. Most chrysanthemum varieties expressed mottling when growing actively, but later it was masked. No flower distortion appeared in 13 chrysanthemum varieties tested; flowers of infected China aster were distorted. Tobacco, petunia, and *Physalis angulata* were best test plants, but not sensitive enough for reliable detection of aspermy virus in chrysanthemum. Four species of aphids transmitted the virus in the non-persistent manner. *Myzus persicae* transmitted it from chrysanthemum to chrysanthemum, tobacco and tomato; from tobacco to tobacco and tomato; and from tomato to tomato. The average efficiency was 50 per cent. *Myzus solani* transmitted it from chrysanthemum to chrysanthemum and tobacco, from tobacco to tobacco, and from tomato to tomato. The average efficiency was 75 per cent. *Macrosiphoniella sanborni* transmitted from chrysanthemum to chyrsanthemum and tobacco; average efficiency was 20 per cent. *Rhopalosiphum rufomaculatum* transmitted from chrysanthemum to chrysanthemum. The average efficiency was 25 per cent. Failure to produce mosaic in cucumber and fleck in Creole Easter lily distinguishes tomato aspermy virus from cucumber mosaic virus.

Moisture Conditions and Control by Heat of the Mushroom-Spawn Nematode, Ditylenchus sp. CAIRNS, ELDON J. Experiments were performed to determine the effects of environmental moisture on the thermal-death responses of the nematodes. A Sibling culture provided nematodes in the state of anabiosis. Equal samples of nematodes were exposed to 3 moisture levels and allowed to reach equilibrium as evidenced by constant weight. One lot was held in water, restoring the nematodes to activity. A second lot was held in a moist-chamber. A third lot was held in a desiccating chamber. The heat treatment involved a gradual approach to and decline from temperatures selected to simulate conditions of heat application in mushroom production. The results prescribe limits in terms of times and temperature necessary for killing under practical extremes of environmental moisture. The amount of moisture determines the mode of lethal action and the degree of resistance to heat. Death of nematodes kept active in water is possibly caused by the melting of a body constitu-

ent. A narrow temperature range (40–45°C.) causes death regardless of exposure time. Death of nematodes in anabiotic state show temperature-time gradients. These gradients vary with moisture level and are at temperatures higher than the critical temperature for active forms. The lower the moisture level, the wider the gradient and the higher the resistance.

Resistance of Sugar Beets to Virus Yellows. COONS, G. H. In an experimental planting at Salem, Oregon, a number of commercial sugar beet varieties and inbreds were being grown to seed. The plants were subjected to virus yellows from a previous sugar beet seed crop, the exposure being extremely severe. This permitted appraisal, in June 1952, of the relative incidence of virus yellows among the cultures. Two dates-of-planting were involved; no contradictions were found between the 2 portions of the test in the readings of disease incidence. The following inbreds were noted as almost free of virus yellows: U. S. 201-B (a leaf spot resistant inbred) and NB6-56, SL 0502, SL 0504, and SL 8255, curly top resistant inbreds. U. S. 216 showed about 25 per cent incidence of virus yellows. U. S. 15, U. S. 225, U. S. 1177, SL 101 (monogerm inbred), and various commercial varieties were all highly susceptible, from 75 to 100 per cent of the plants being affected. Plantings of the 4 curly top resistant inbreds at Salinas, California, gave readings for virus yellows incidence in line with the Oregon results. Virus yellows resistance in sugar beets has not been demonstrated in Europe in spite of a large amount of selection work, only tolerant varieties being reported. The finding of resistance to virus yellows in inbred lines may afford a new approach to control of this disease.

Effect of Clean Fallow in Root Knot Development on Soybeans. CRITTENDEN, H. W. Field experiments in Delaware in 1951 indicated that root knot nematodes were reduced as a result of clean fallow of the soil during June. Field experiments in 1952 were designed to check these results. All ground was prepared on May 1 and certain plots were kept in clean fallow by cultivation once every 2 weeks from May 1 to time of planting. Dates of planting soybeans were May 15, June 1, June 15, and July 1. Seed was sown in rows 6 in. apart; no cultivation occurred following planting date. The root knot indices (100—maximum infection) obtained from examination of the roots of 90-day old plants from plots planted at different dates were: 50 May 15; 56 June 1; 17 June 15; and 4 July 1. Plots not in clean fallow in May and June were planted to soybeans on July 1, and the root knot index of these plants after 90 days was 40. These experiments, using soybeans, indicate that the root knot nematodes in the top 6 in. of soil were greatly reduced as a result of clean fallow and cultivation of a loamy sand during June.

Production by Basidiobolus spp. of odor familiar in Streptomyces spp. and in benzene hexachloride. DRECHSLER, CHARLES. A species of *Basidiobolus* (possibly *B. haptosporus*) with zygospores of undulate profile, which occurs widely in leaf mold, gives off a strong "earthy" odor similar to that emitted by many species of *Streptomyces* (including *S. scabies*) and by the commercial insecticide benzene hexachloride. The same odor is produced in maize-meal agar cultures, also by an obviously different fungus supplied as *B. ranarum* to the American Type Culture Collection by the Centraalbureau voor Schimmelcultures of Baarn, Holland. A species of *Basidiobolus* with smooth zygospores which occurs especially in water-logged forest detritus may have some relation to the earthy odor often noticeable in wet areas. An unpleasant musty smell of fish that has been ascribed wholly to the Actinomycetes may perhaps originate also from *Basidiobolus* spp. That submergence in water may favor odor production is indicated by experience with many root-rotting oomycetes. Thus, when *Pythium debaryanum* or *P. ultimum* is grown on unwet maize-meal agar no odor is evolved; but when slabs of maize-meal agar, well permeated with mycelium of these terrestrial species, are transferred to sterile water an odor much like the wholesome odor of newly caught fresh-water fish becomes noticeable after several hours.

Two New Nematode-capturing Fungi. DRECHSLER, CHARLES. A species of *Arthrobotrys* producing uniseptate elongate-obovoid conidia, usually about 36μ long and 18μ wide, in a single terminal cluster, captures eelworms through contraction and closure of 3-celled rings generally similar to the predacious rings of *A. dactyloides*. All 3 arcuate cells composing the individual ring participate directly in constricting a captured animal. Each ring is borne on a stout 2-celled stalk whereof the proximal component cell is usually nearly equal in length and width. A species of *Dactylella* with spindle-shaped quadriseptate conidia commonly about 50μ long and 15μ wide captures eelworms exclusively by adhesion to elongated-ellipsoidal cells about 7μ long and 5μ wide. These cells are not supported on stalks like the similar adhesive organs known in several related nematode-capturing species, but are borne directly on the mycelial hyphae. They contain numerous subspherical granules, thereby differing further from the stalked globose predacious organs found elsewhere, which usually are filled throughout with protoplasm of nearly homogeneous appearance.

Further Results on Testing Susceptibility of Pear Seedlings to Erwinia amylovora. DUNEGAN, J. C., H. H. MOON, AND R. A. WILSON. On April 29 and May 12, 1952, pear seedlings (961) of known parentage were inoculated with *Erwinia amylovora*. The inoculum, as in 1951, was applied with a power sprayer and gun to drench 1 side of each tree with a suspension of the bacteria and pyrophyllite. Blight symptoms developed on 546 (57 per cent) of the seedlings and 352 (37 per cent) were so severely infected that they were removed by mid-summer. In 1951, when 900 (52 per cent) of the original 1732 seedlings were infected, only 324 (19 per cent) had to be removed. This difference is believed to reflect the more favorable environmental conditions that prevailed in 1952 for the development of *E. amylovora* after the seedlings were infected.

Some Effects Resulting from the Introduction of Antibiotics Into Fruit Trees. DUNEGAN, JOHN C. AND R. A. WILSON. Chlorotic symptoms developed on leaves of Bartlett pear seedlings 10 days after they had been sprayed with a solution containing 500 p.p.m. of streptomycin. Striking chlorotic patterns developed on peach leaves 14 days after a gelatin capsule containing 50 mg. of streytomycin had been inserted in the trunk of a young Elberta peach tree. A 7-year-old Elberta peach tree developed a yellow-green color following the injection, over a period of 84 hours, of 28.4 liters of a solution containing 1.7 grams of terramycin. Subsequent leaf drop due to *Xanthomonas pruni* infections was much less from the terramycin-treated tree than from the control trees injected with water.

Surveys for Larch Canker. FOWLER, MARVIN E. A resurvey for European larch canker was initiated in September 1952 with funds provided by the Forest Pest Control Act. The disease, caused by the fungus *Dasyscypha willkommii*, was first discovered in America in 1927 on European larch. The infected trees had been imported from Great Britain early in this century and planted in Essex County, Massachusetts. Following surveys, from 1927 to 1938, 3700 infected trees, mostly European larch (*Larix decidua*) and a few Japanese larch (*L. leptolepis*), were cut and burned in an attempt to eradicate the disease before it spread to native larch stands. The disease was not found on native larch (*L. laricina*) near infected plantations in Massachusetts or on exotic larch elsewhere. The current survey has led to the discovery of several old infections in 1 small European larch plantation in Essex County that was overlooked in the earlier surveys. In addition, several young cankers have been found on 3 European larch trees in another plantation. All these infected trees were cut and burned. The occurrence of the disease on so few of the thousands of trees inspected so far indi-

cates a high degree of success in the previous attempts to eradicate the disease.

The Status of Oak Wilt. FOWLER, MARVIN E. Surveys conducted during 1952 by Federal, State, and other agencies, did not extend the known range of oak wilt beyond the 18 States in which the disease was previously known. However, the disease was found in numerous additional counties. A number of States are conducting surveys and control programs. The best control that can be recommended now is to destroy the oak wilt infected trees to remove them as hazards in the spread of the disease. Diseased trees should be cut and burned or utilized promptly. Before a control program is initiated, consideration should be given to the following: 1) in those areas where oak wilt is well established and infection centers are large, a suppression program would be costly. The oak values in some of these areas are not high and the cost of eradicating diseased trees might be excessive. 2) In the Ozarks and throughout the East the percentage of diseased trees is exceedingly small and oak values are high. The cost of eradicating all the oak-wilted trees would be insignificant in comparison with the value of the residual oak forests.

A Root-knot Nematode Attacking the Crown, Petiole, and Leaf of African Violet. GOLDEN, A. MORGAN. Root-knot nematodes are a major problem in growing African violets (*Saintpaulia ionantha* Wendl.). Control measures that should eliminate root parasitic nematodes have appeared ineffective against root knot in this host. Recent observations have furnished a possible cause for their inefficiency. They have revealed that a root-knot nematode of the *Meloidogyne arenaria* group occurs not only in the roots but also in the crowns, petioles, and leaves of African violets in contrast to most other plants. All developmental stages of the parasite were found in the infected green parts and infection occurred along the vascular tissues. Giant cells were always formed. The crowns and petioles exhibited slight swellings to heavy thickenings, even to distortions in heavily infected crowns. The leaves were thickened in spots, and in instances had blister-like galls, the symptoms increasing with the development of the nematode. Because of the invasion by the root-knot nematode of crowns, petioles, and leaves of this plant, propagation by crown separation and leaf cuttings appears to have perpetuated the infection. It is recommended that only nonnematized plants be used for propagation, or in case of infected plants to use only leaves that are free of infection.

Binomial Classification of the Chrysanthemum Stunt Virus. KELLER, JOHN R. The chrysanthemum stunt virus has been classified according to the Holmes' binomial system in the tobacco mosaic group of the Marmoraceae. The name *Marmor chrysanthemi* has been proposed. This classification is based on the method of transmission and on physical properties of the virus. The virus can be transferred by juice or dodder (*Cuscuta gronovii*), but no insect vector is known. The 3–4 month incubation period for stunt virus symptom expression after inoculation can be reduced to 6–8 weeks by keeping the growing point defoliated above the inoculation site for 4 weeks. The virus does not move out of the inoculated leaves in sufficient quantities to establish infection in other parts of the plant in less than 35–45 days after inoculation. The stunt virus was found to have a thermal inactivation point above 96° C. and below 100° C. It is infectious in dilutions up to 10^{-4} and remains infectious at least 2 years in dried tissue. Infectivity is retained for at least 100 days at 3° C. *in vitro* but is lost after 55–60 days at 21° C. The virus withstands freezing *in vitro* for at least 1 year.

A Spot Anthracnose Disease of Bouchea in Cuba. KEVORKIAN, ARTHUR G. A new spot anthracnose or scab disease of a common roadside verbenaceous weed, *Bouchea prismatica* (L.) Kuntz was discovered in Las Villas province in Cuba. The causal organism is *Elsinoe boucheae* n. sp. of the Myriangiales. The lesions are usually prominent on the upper portion of the stem but in severe cases they may occur on the entire stem. The roundish or slightly elongated lesions have raised, well delimited brown to black margins, with either plane or slightly depressed, dirty white centers, measuring 0.5–4.0 × 0.4–2.0 mm. Leaf lesions are practically confined to the lower surface of the midrib and veins and appear as darkish swellings that are raised and sometimes have a colored center, 1.0–3.0 × 0.2–0.5 mm. Ascomata roundish, irregularly scattered on the surface of the lesions, developed on the outer layer of a well developed hyaline prosenchyma, up to 80 μ thick, made up of a hyaline or slightly yellowing pseudoparenchyma, covered with a well differentiated epithecium, composed of 2–4 layers of dark, flattened cells; 80–120 × 30–45 μ. The asci are globose, 16–18 μ in diameter, containing up to 8, triseptate, hyaline ascospores measuring 10–12 × 3–5 μ. Inasmuch as the specific host plant is a roadside weed no control measures were deemed necessary.

Retention of Virulence of Lyophylized Cultures of Pseudomonas caryophylli. MILSTREY, R. A., R. N. STEWART, AND W. F. JEFFERS. *Pseudomonas caryophylli* Burkh. usually loses virulence quickly in culture. Since a virulent organism must be used for artificial inoculations in breeding for resistance to bacterial wilt of carnations, various media and methods of storing were employed in an attempt to retain pathogenicity. These were beef-peptone agar kept under mineral oil, beef-peptone agar frozen at —15°C.; carnation infusion agar under oil and carnation infusion agar frozen; a so-called super agar containing vitamins, peptone, beef extract, dextrose, and carnation extract; and lyophylization. Suspensions of bacteria in beef-peptone broth were prepared from cultures treated as indicated. After inoculation by a 1-minute dip in the bacterial suspension, rooted cuttings were potted and bottom heat applied. After 8 months only the lyophylized culture and the culture on carnation infusion agar under oil caused more plants to become wilted than did the check culture. All other cultures apparently were avirulent or non-viable. Noninoculated plants used as controls often became infected when randomized with inoculated plants. Nevertheless, plants inoculated with the lyophylized culture or the culture maintained under oil on carnation-infusion agar showed a much higher percentage of wilt than control plants.

Experiments With Fungicide-insecticide Dust Mixtures on Outdoor Roses. SMITH, FLOYD F., PHILIP BRIERLEY, AND W. R. JENKINS. Thirty dust mixtures were applied 17 times at weekly intervals in 1952 to 4 replicated plots each containing 5 varieties of roses. Differences between treatments in stem length, number, and weight of flowers produced were greater late in the season than earlier. The fungicides included sulfur 65 or 25 per cent, alone or mixed with ferbam 10 per cent or copper oxysulfate 3.4 per cent; manganese ethylene bisdithiocarbamate (Manzate) 10 per cent; or captan 10 per cent. A miticide—Aramite (88R) 1.5 per cent, p-chlorophenyl p-chlorobenzene sulfure (Ovotran) 1.5 per cent, or malathon 4 per cent—was mixed with each fungicide. The insecticides DDT 5 per cent and lindane 1 per cent were in all mixtures. Of the fungicide dusts applied without miticides Manzate was best, captan or ferbam-sulfur were slightly inferior, and sulfur with or without copper oxysulfate was poorest. Adding Aramite to Manzate resulted in greater flower production but adding malathon gave fewer and smaller flowers. Aramite in ferbam-sulfur mixture gave fewer and smaller flowers whereas malathon gave increases comparable to Aramite-Manzate mixtures. Aramite or malathon used with captan gave higher flower production than malathon-ferbam-sulfur or Aramite-Manzate. All fungicides controlled blackspot but none controlled mildew.

Control of post-harvest brown and rhizopus rots of peaches. SMITH, WILSON L., JR. AND MARK H. HALLER. Sixty materials were tested in post-harvest treatments of hard to firm peaches of several varieties from different growing areas. Inoculation was done by spraying unin-

jured peaches with spore suspensions of *Monilinia fructicola* (brown rot fungus) or by spraying injured peaches with or dipping them in spore suspensions of *Rhizopus nigricans*. Selection of promising materials was based on reduction of decay at least 50 per cent below that in checks and complete or almost complete absence of fruit injury or off-flavor. Spraying with Dowicide A (0.5 per cent) reduced brown rot 67 per cent and rhizopus rot 57 per cent. Orthocide 406 (1 per cent) and Isothan Q 15 (0.5 per cent) reduced brown rot 76 and 75 per cent, but were ineffective against rhizopus rot. Fumigation immediately after inoculation with trichloroethylene (1:20,000) or tetrachloroethylene (1:20,000) reduced *Rhizopus* infection 58 per cent, but was ineffective against *Monilinia*. Fumigation delayed 24 hours was ineffective against both organisms. S. tetrachloroethane and 1,1,2 trichloroethane were ineffective against both organisms and caused off-flavors. *Rhizopus* caused more decay of uninoculated fruit than *Monilinia*. *Rhizopus* infections were reduced 82 per cent and *Monilinia* infections only 28 per cent by wrapping peaches in copper-impregnated paper (Hartman wraps).

Longevity in Ustilago nuda. TAPKE, V. F. Chlamydospores of the "nuda" or floral-infecting loose smut fungus of barley normally retain high viability only for a few months when stored at room temperature. This is a serious obstacle to progress in a study of physiologic races of the species. It has been found that viability may be greatly prolonged by storing the chlamydospores at 28°–32° F. Some results of storage at these temperatures are: 1) good germination has been obtained in numerous *U. nuda* collections 1–10 years old, 2) a 13-year old and a 14-year old collection germinated 14 and 12 per cent respectively on 2 per cent potato-dextrose agar and 3) spores of the 14-year old collection were used to inoculate flowers of Alpha (C. I. 959) barley under pure culture conditions. Seed from these flowers produced plants smutted with *U. nuda*. Seed from barley flowers inoculated with highly viable "nuda" collections also has been stored at 28°–32° F. Up to 73 per cent of smutted heads has been obtained in plants from such seed after 7 years of cold storage.

Physiologic Races in Ustilago nuda. TAPKE, V. F. Studies to date confirm the reports of previous investigators that barley varieties for differentiating physiologic races of *Ustilago nuda* (Jens.) Rostr. are difficult to find. In tests involving 57 spring varieties only 3 types of usable differential action have been isolated i.e. the Charlottetown (C. I. 2732) Compana (C. I. 5438) and Valki (C. I. 5748). Stephan (C. I. 8051) though similar to Compana may represent a fourth type. One hundred and twenty three "nuda" collections from 31 states have been tested on these 4 barleys. Five distinct races have been isolated. One race that is widespread but more prevalent in the north than in the south, occurred in 60 per cent of the collections. Another race common in the south occurred in 26 per cent of the collections. The remaining 3 races combined occurred in only 14 per cent of the collections. In previous studies by the writer of physiologic races of barley covered smut (*U. hordei*) and "nigra" loose smut (*U. nigra*), 1 race likewise occurred in more than half the total number of collections of each species.

Botryosphaeria Rot of Apple in Maryland. WEAVER, L. O. Fruit rots of apple appearing in August 1952, caused considerable pre-harvest and post-harvest loss of fruit in Maryland. The organisms involved were *Botryosphaeria ribis* G & D and *Physalospora obtusa*. The 2 diseases are difficult to diagnose from symptoms in the field. Botryosphaeria rot is characterized by 1) a soft, mushy texture of diseased tissue; 2) a scalded appearance of the fruit surface with lack of pigment in red varieties; 3) drops of liquid on the rotted area. From field observations and limited inoculation tests, the varieties Golden Delicious, Rome Beauty, Grimes and Northwestern Greening are most susceptible. The imperfect stage of each fungus was found on dead wood in the orchards. In 1 case, *Dothiorella* sp. was found associated with cankered and dead branches on Rome Beauty. Abundant inoculum and above-normal rainfall in harvest period may have been important factors in the development of Botryosphaeria rot.

Preservation of Cultures of Microorganisms under Oil Seal. WEISS, FREEMAN AND BAKIR A. OTEIFA. Although the use of a paraffin oil seal on agar cultures is now almost a routine practice in the maintenance of stocks of mircoorganisms, questions still arise as to whether a particular genus or species will survive this treatment. In the last 4 years various molds and other filamentous fungi, yeasts, streptomycetes, and bacteria in the American Type Culture Collection have been preserved by this method. The proportion of recovery has been high—about 96 per cent of 1244 strains that were oiled in 1951 were recovered after 12–18 months. Individual examples of survival for 3–5 years have been noted. A more extensive list of genera of fungi, successfully preserved by this method, than has heretofore been published is presented. Certain genera and species have consistently failed to survive, but their number is small. No tests have been made of the retention of physiological characteristics, including pathogenicity, but very few of the cultures show new growth activity under oil, especially when refrigerated. The method is a valuable supplement to lyophilization, since it can be applied to nonsporulating cultures.

Field Control of Halo Blight of Beans with Streptomycin. ZAUMEYER, W. J., H. R. THOMAS, J. W. MITCHELL AND H. H. FISHER. Topcrop and Bountiful beans were sprayed 1–4 times with a 0.1 per cent aqueous solution of pure streptomycin sulfate plus 0.3 per cent Tween 20 as a spreader. Treated control plots were sprayed 4 times with Fermate or Orthocide. The first application was made when the simple leaves were about ¾ expanded and the others at weekly intervals. Three days following the first application, the plants were inoculated with *Pseudomonas phaseolicola*. Of an average of approximately 700 plants in the 4 replicates of each treatment, 41 per cent were infected in the plots receiving 1 application of streptomycin, 10 per cent in those receiving 2 applications, none in those receiving 3, and a trace in those receiving 4. In the untreated plots, 93 per cent of the plants were infected. In the plots sprayed with Fermate or Orthocide, 88 and 91 per cent infection, respectively, were noted. Of an average of about 900 pods produced in the 4 replicates of each streptomycin treatment, the following percentage infection was found: 1 spray, 19; 2 sprays, 12; 3 sprays, 3; and 4 sprays, 1. Pods from plants sprayed with Fermate or with Orthocide showed 18 and 20 per cent infection, respectively, while 19 per cent of the pods on the untreated checks were infected.

LISTING OF BOOKS

PHYTOPATHOLOGY will, from time to time as space becomes available, list titles of books on phytopathological subjects. A brief description of the contents of the books will be included.

BAWDEN, F. C. 1950. **Plant viruses and virus diseases.** Ed. 3. Chronica Botanica Company, Waltham, Massachusetts. 335 pp., price $6.00.

This completely rewritten edition of a well-known text contains one new chapter and many new illustrations.

ELLIOTT, CHARLOTTE. 1952. **Manual of bacterial plant pathogens.** Ed. 2. Chronica Botanica Company, Waltham, Massachusetts. 186 pp., price $6.00.

In this revised edition, each bacterial plant pathogen is listed with its synonyms, its cultural, morphological, and physiological characteristics, disease symptoms produced, geographical distribution, and methods of control. Important literature citations are also included.

PRESTON, R. D. 1952. **The molecular architecture of plant cell walls.** John Wiley & Sons, New York. 211 p.

The author deals with those aspects of cell-wall studies of greatest appeal to botanists. The first few chapters give a brief resume of the more important physical and chemical approaches to cellulose structure; the remaining chapters are concerned with the detailed architecture of cell walls in a variety of plants, and attempt to interpret growth processes in terms of the structure thus revealed.

WAKSMAN, S. A. 1952. **Soil microbiology.** John Wiley & Sons, New York. 356 p.

The author presents a broad outline—a philosophy—of soil microbiology. Included are discussions of the soil microbiological population, the general fauna and flora of the soil, mutual interrelationships among microorganisms, decomposition of plant and animal residues, formation of humus, transformation of various elements essential for plant growth, and applications to soil formation and plant nutrition.

PAINTER, R. H. 1951. **Insect resistance in crop plants.** The MacMillan Co., New York. 520 p.

This book deals with the general principles of control of insects through host-resistance, and brings together in one volume references to the literature on the subject. Equal emphasis is given to entomological and plant-breeding phases. Specific chapters deal with resistance to insects in wheat, corn, cotton, sorghums, and potato